

Historic, Archive Document

Do not assume content reflects current
scientific knowledge, policies, or practices.

80.39
315
y.2

Do not put in file

-

PROGRESS REPORT
of the
EASTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1967, and March 31, 1968. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

July 1, 1968

U. S. DEPT. OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY

JAN 27 1969

CURRENT SERIAL RECORDS

TABLE OF CONTENTS

	Page
Introduction.....	i
Dairy Utilization.....	1
Meat Utilization.....	19
Animal Fats and Oils Utilization.....	28
Hides and Leather Utilization.....	37
Potato Utilization.....	48
Vegetable Utilization.....	54
Deciduous Fruit and Tree Nut Utilization....	57
Tobacco Utilization.....	61
Maple Sap and Sirup Utilization.....	72

INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is to develop new and expanded markets for designated farm commodities, through research to develop new and improved products and processes based on these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup and Eastern fruits and vegetables including potatoes.

In carrying out its mission, the Division does research in physical and biological science and in engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research. When appropriate, engineers conduct pilot plant studies of promising laboratory developments to provide engineering and cost data essential to industrial application feasibility determinations.

The research effort at the Eastern Division amounts to approximately 183 scientist man-years. In addition, the Division supervises domestic research contracts and grants equivalent to 35.2 scientist man-years per year at 32 locations in the United States. The Division's program is supplemented by a variety of research projects in foreign countries under PL-480 grants. There are now a total of 32 such grants in 11 countries.

The Division is organized in 10 laboratories of which 5 are located entirely at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and one is at Washington, D. C., with part of its research program at Beltsville. One of the laboratories at Wyndmoor has some of its research located at East Grand Forks, Minnesota, and a second has research located at Lexington, Kentucky. One of the Division's laboratories is devoted to pioneering research on the biophysical mechanisms of macromolecules, and is located at Brandeis University, Waltham, Massachusetts.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, state experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 8 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts; the major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. It is thus evident that maintaining and enlarging the markets

for these and the other farm products under study at the Eastern Division should be a major national concern.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Following are some examples of recent developments based on research at the Eastern Division and on Division-supervised research that typify such achievements.

Examples of Recent Accomplishments of the Eastern Utilization Research and Development Division

Modifying Cigarette Smoke Composition. As part of the U. S. Department of Agriculture's cooperative program on tobacco and health, the University of Kentucky has been determining the effect of chemicals on modifying smoke composition. Cigarette tobacco is being treated with chemicals such as flame retardants, free-radical scavengers, oxidants and temperature depressants. Some changes in the levels of possible health-related smoke constituents have been detected. Decreases of 27% for total particulate matter (tar); 42% for nicotine; 34% for phenol and 71% for benzo(a)pyrene were obtained. The smoke from the treated tobaccos will also be assayed biologically on laboratory animals to determine if any correlation exists between the decreased levels of the selected smoke components and the tumorigenicity of the smoke. The development of this information will be a major step in selecting additives of practical importance.

Milk Storage in Nuclear Emergencies. USDA-sponsored contract research at Pet Inc. has proved that commercially pasteurized milk can be stored long enough to permit the decay of radioactive iodine contamination to safe levels. Iodine-131 is transmitted to milk in significant amounts from transitory fallout. Because its half-life is 8 days, the radioactivity almost disappears within 40 days. But at current commercial storage conditions of 45°F., the milk flavor and bacteriological quality deteriorate before the iodine-131 can decay sufficiently. This research has shown that a 32°F. storage temperature extends the shelf-life fivefold to about 4-1/2 weeks. Also, milk which has been pasteurized by ultra-high-temperature methods and refrigerated at 32°F. will keep for as long as 20 weeks. Aimed primarily at extending our milk supplies in nuclear emergencies, this research is also of immediate practical importance to dairy manufacturers and retailers. It emphasizes the advantages of lower storage temperatures in increasing product shelf-life and in reducing loss due to spoilage. The same advantages may also apply to other dairy products, such as cottage cheese.

Market Potential Established for Vacuum Foam-Dried Whole Milk. The prospects for the commercial success of the beverage quality vacuum foam-dried whole milk developed by Department engineers appear promising according to early results from the market testing in nine supermarkets in the Philadelphia suburbs. The product was priced at 4 cents less per quart than fresh whole milk. In a 12-week period, approximately 280 cases of 24-quart-equivalent

packages were sold--a very high rate of average sale per store for any new item. A substantial number of the purchases were made to supplement the customer's fresh milk supply rather than to replace it. Data on the consumer evaluation phase are in the analysis stage, but preliminary indications are that almost 60% of the adults and more than 80% of the children liked the reconstituted milk in all respects. Repeat purchase patterns are being determined from follow-up interviews with many of the buyers.

New Cheese Whey-Soy Flour Combinations Developed for Food Use. Standard dairy plant equipment has been adapted to the manufacture of easily dispersible powders from cheese whey solids and soy flour. In this process the soy flour is dispersed into either acid or sweet cheese whey, pasteurized, homogenized, condensed under vacuum and spray-dried. On reconstitution, the product composition can be varied with the addition of flavors, sweeteners and either vegetable or animal fats. During the last year a variety of new whey-soy products intended for human and animal use were informally evaluated by interested parties in UNICEF, industry and certain universities. The encouraging results affirm the belief that whey-soy blends can afford a practical means for adding to the world's food supply and increasing U. S. farm income. And, with this promising new outlet for surplus cheese whey, the water pollution load from the dairy industry could be reduced substantially.

Ultraviolet Pasteurization of Apple Cider. Farm-fresh apple cider can now retain that "straight-from-the-press" flavor longer if exposed to germ-killing ultraviolet irradiation during processing. Department scientists developed a simple process which increases the refrigerated shelf-life by several weeks because 95% or more of the spoilage microorganisms normally found in fresh cider are destroyed. In contrast to older chemical preservative methods, this process does not affect the flavor, since nothing is added to the cider. The process is intended for relatively small operations and should help expand markets for grower-produced cider by safeguarding the natural apple cider flavor through longer transportation and storage periods.

Reactive Chemical from Surplus Animal Fats. A new chemical has been developed that permanently incorporates fatty acids into a wide variety of materials, building in properties such as lubricity, oil solubility, and waterproofness. In the first step of the process, animal fats are converted into certain esters that on heating liberate the fatty acid in a highly reactive form called a fatty ketene. The latter permanently bonds the fatty acid to the substance chosen. The ketene from stearic acid can attach fatty stearyl groups to drugs such as barbiturates and sulfanilamides, to sugar for detergents, to sulfur, phosphorous and silicon chemicals. It can serve as a sizing agent for papermaking, or be used to make waxes and lubricants. Researchers are exploring its possibilities for treating leather, cotton, wool and other textiles to impart lasting water repellency and softness.

Glutaraldehyde-tanned Leather In Combat Gear. To meet the need for rugged and durable combat footwear, the military services designed a boot with an upper made partly of leather and partly of nylon fabric. The latest improvements in leather properties make these boots more resistant to wetting by water and to deterioration from perspiration. To develop these new leathers, industry adopted the results of research conducted at the Department's Eastern Utilization Research and Development Division. This research demonstrated that the new tanning agent, glutaraldehyde, not only confers on leather exceptional resistance to deterioration from perspiration, but also makes treatment with water repellent agents more efficient. The improved leathers are also being used for work shoes, sporting boots and other civilian footwear.

Fat Analysis Technique Improves Sausage-making Technology. Department meat researchers have shown that differential thermal analysis can be advantageously used to predict the melting characteristics of fats in sausage formulations. In spite of the recent technical advances in sausage-making, the fat ingredients are still incorporated on a "rule of thumb" basis, and the different qualities of meat fats greatly influence the processing behavior and the product quality. This practice leads to one of the most serious problems in the industry--the tendency of fats to be released under certain conditions, especially during the cooking step. The differential thermal analysis technique detects variations in the melting patterns of different types of animal carcass fats. These data can be directly related to optimal product formulation, processing conditions and product quality and are expected to lead to further improvements in sausage-making technology.

DAIRY UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968				
	Research Problem Area				Total
	410	601	702	901	
Pennsylvania (Wyndmoor)	:	:	:	:	:
Maryland (Beltsville)	:	:	:	:	:
District of Columbia	:	:	:	:	:
A. Chemical Composition,	:	:	:	:	:
Physical Properties	:	:	:	:	:
and Structure	25.9	0	0	0	25.9
B. Flavor	5.6	0	0	0	5.6
C. Color, Texture and Other	:	:	:	:	:
Quality Factors	1.3	0	3.4	0	4.7
D. Microbiology and Toxicology:	2.9	0	1.7	0	4.6
E. Technology - Process and	:	:	:	:	:
Product Development	13.2	1.7	0	0.5	15.4
Total	48.9	1.7	5.1	0.5	56.2

Intramural program is supplemented by extramural support representing (a) 3.6 SMY's at universities and state agricultural experiment stations^{1/}, (b) 0.5 SMY's at other U.S. institutions^{2/}, and (c) PL-480 funds in 8 countries representing 1,013,041 U.S. dollars equivalent.

1/ RPA 410 - Chemical Composition, Physical Properties and Structure, 0.5; Flavor, 2.3; Technology - Process and Product Development, 0.8

2/ RPA 702 - Chemical Composition, Physical Properties and Structure, 0.5.

Problems and Objectives

The milk industry in the U.S. is characterized by a surplus of butter, non-fat milk, and cheese whey. Excess butter and nonfat milk are purchased by the Government for school lunch, welfare, and foreign distribution programs. Cheese whey is a serious stream pollution problem. Research on utilization of milk emphasizes the development of better processing procedures, improved dairy products, new and increased food uses for those milk components in excess supply, and more economical disposal of dairy wastes for which there is no profitable use.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Develop dry whole and concentrated milks of beverage quality.
2. Prevent the formation of or eliminate objectionable flavors in dairy products, and stabilize and intensify desirable flavors.
3. Prevent or eliminate contamination of dairy products with disease-producing microorganisms.
4. Develop food uses and more economical disposal processes for cheese whey.
5. Develop new cheese and milk fat products for food use.

Progress - USDA and Cooperative Programs

RPA 410 - NEW AND IMPROVED MEAT AND MILK PRODUCTS

A. Chemical Composition and Physical Properties

1. Milk Protein Composition and its Relation to Properties of Concentrated and Dry Milks. During separation and purification of proteins by acrylamide gel electrophoresis some proteins, as β - and γ -caseins, are changed chemically by free radicals generated in the electrophoresis process. Thus, the limitations of the method in establishing homogeneity or heterogeneity must be recognized. Recognition of γ -casein as a distinct component of cow's milk casein arose largely because it has fewer phosphate groups than the more abundant α_{s1} - and β -caseins. Comparison of polymorphic forms of γ - and β -caseins now indicates that pairs of polymorphs have identical amino acid differences. This implies identical base substitution in the casein gene through mutation and presumptive common ancestry of γ - and β -caseins.

Attempts to account for the reactivity of milk proteins and their tendency toward aggregation are severely hampered by ignorance of the proteins' primary molecular structure and the precise form of their molecules. Progress can be made by comparing differences in behavior induced by specific reaction of small numbers of particular amino acid residues within

the protein molecule. Lysines of the Ca-sensitive α_{s1} -casein (Cn) were progressively modified, while retaining charge characteristics, by guanidination, and, with loss of charge, by carbamoylation. Completely guanidinated α_{s1} -Cn no longer precipitated with calcium ion. The protection offered by κ -casein to precipitation of α_{s1} -casein by calcium ion did not change until the 5th (out of 9) lysines per molecule of κ -casein was reacted. At this point carbamido κ -casein completely lost its stabilizing power; guanidinated κ -casein lost 30%. Complete loss attended guanidination of 2 additional lysines, both in the para- κ -casein part of the molecule. Measurements of circular dichroism detected no difference in molecular form resulting from chemical modification. Full understanding of the mechanism of the stabilizing reaction remains the goal of further experiments. The ability of β -lactoglobulin to specifically bind sodium dodecyl sulfate is lost on mild photooxidation that specifically reacts with histidine and tryptophan. κ -Casein has the unique ability to protect the major caseins, α_{s1} and β , from precipitation by calcium ion in milk. κ -casein's stabilizing power was unaffected by heating 5 minutes at 100°C. in water solution, but was considerably impaired if sodium chloride were present, and was lessened even more by reducing agent plus salt. Nitration of κ -casein tyrosyls brought progressive loss of stabilizing power. Ease of complete nitration infers lack of tight secondary and tertiary molecular structure and ready accessibility of all tyrosine side chains for reaction. κ -Casein that was functionally destroyed by rennin cleavage into para- κ -casein and macropeptide (MP), has been functionally restored by coupling para- κ -casein and thiolated MP. In β -casein secondary phosphate ionization is critical for precipitation by calcium ion. Aggregation of acylated β -casein molecules increases with length of the alkyl chain, doubtless due to hydrophobic bonding. Acylated β -caseins are still stabilized by κ -casein against precipitation by calcium ion. From the viscosity of β -casein solutions it was deduced that β -casein molecule is essentially devoid of any regularity in its structure. Analytical procedures have been developed for the use of antibodies to the caseins for assessing homogeneity, aggregation, and chemical alteration.

Size and form of casein micelles were determined by electron microscopy. Casein micelles from fresh, freeze-dried and spray-dried skim milks were equivalent. In 2:1 high-temperature short-time sterilized concentrated skim milk the casein micelles were twice as large. With added polyphosphate there was no aggregation. Using ferritin-labeled antibodies to κ -casein, the attempt was begun to locate the protective colloid, κ -casein, in the casein micelles. Two avenues - construction of artificial casein micelles from the separated casein components, and specific spatial labeling of caseins in natural micelles - should result in better description of the casein micelles which determine so largely the textural properties of milk. Solvation of ultracentrifuged skim milk varied with breed, and Ca:P ratio in the κ -casein. Solvation was in roughly direct proportion to heat stability of the milk. Automatic fat globule size counting was applied to reconstituted vacuum foam dried milks and the data correlated with occurrence of unwanted scum. Technique shows promise for quality control in further developing the drying method. Structure of milk fat globule membranes is

being examined by electron microscopy.

In contract research at the University of Minnesota efforts to relate the heat stability of individual milks to genetic variants in cows were unsuccessful. Why some milks are more resistant than others to heat coagulation is still an unsolved problem.

In PL-480-supported research at the University of Uppsala, Sweden, on the development of methods for purification of protein complexes, additional examples of the preparation of specific adsorbents are reported. Blood group A substance was coupled to agarose; phytohemagglutinin was coupled to agarose and to Sephadex G-25. Since blood group A substance and phytohemagglutinin strongly associate, each of the specific adsorbents could be used to adsorb the other compound (pH 7.5). Desorption was accomplished in 1 molar sodium chloride at pH 5.0.

In PL-480-supported research at Israel Institute of Technology, Haifa, Israel, a satisfactory method for separating and quantitating a complex mixture of proteins by means of gel electrophoresis and radioactive labeling of proteins has been worked out.

In PL-480-supported research at the University of Graz, Austria, deoxyribonucleic acid was degraded by ultrasound. With the help of the small-angle-X-ray method, it was shown that an elongation of the deoxyribonucleic acid molecule occurs during the addition of actinomycin.

In research at the Indian Institute of Science, Bangalore, India, supported by PL-480 funds, complete elucidation of the mechanism of phosphorylation during biosynthesis of casein is not yet possible, but progress is being made. The bulk of evidence thus far available suggests that phosphorylation follows synthesis of the complete polypeptide chain.

In research supported with PL-480 funds at the National Dairy Research Institute, Karnal, Punjab, India, by modified starch gel electrophoresis showed that, in the proteose-peptone fraction of milk and dairy products (a) the molecular weight of constituents decreases in size gradually in the post partum period; (b) the concentration of the proteose-peptone fraction increases during periods of environmental stress on cows due to climatic conditions; (c) the amount of proteose-peptone goes through a maximum on the heating of milk; (d) the degradation of whey proteins on heating seems to account for the observed maximum; (e) a similar maximum in the proteose-peptone content of cheese was noted during ripening; (f) proteose-peptone in high concentration seems to speed color development and age thickening in evaporated milk.

In PL-480-supported research at the College of Agriculture in Olsztyn, Poland, studies were carried out on the extracellular enzymes liberated by strains of lactic acid bacteria. Strains of Streptococcus lactis were found to have an optimum activity at pH 6.8 on isoelectric casein whereas Lactobacillus casei had an optimum pH of 5.5. Both proteases and

peptidases were demonstrated in the enzymatic extract of one particular strain of S. lactis. Marked differences were noted in the fat hydrolysis, glyceride and free fatty acid composition of cheese during ripening. Blue cheese had more of the higher fatty acids while Romadour cheese contained more of the volatile short-chain fatty acids.

2. Effect of Feed on Milk Fat Composition. Contract research at the University of Maryland has shown that feed composition can influence the composition of milk fat. Corn silage, dehydrated and pelleted, as the total diet produced milk fat with more unsaturation than was formed on a ration containing some grain.

In PL-480-supported research at the Biochemical Institute, Helsinki, Finland, feeding trials demonstrated that protein-poor feeds, when supplemented with adequate amounts of urea, can be successfully utilized for milk production. Furthermore, it has been shown that hemicellulose, a waste product of the wood industry can replace up to 30% of the total feed of cows when sufficient amounts of urea are included in the diet. A bitter off-flavor is occasionally present in the milks of cows fed hemicellulose, the origin of which is under investigation at the present time. Studies on various constituents of the milk and blood of test cows and cows on normal rations are continuing.

B. Flavor

These flavor studies provide basic information needed for the development of improved concentrated and dried milks, better products containing milk fat, and more tasty and uniform cheeses.

1. Control of Oxidative Flavors. In preliminary contract experiments at the University of Maryland, feeding cows rations containing naturally high levels of tocopherol or added tocopherol produced milk with increased resistance to copper-induced oxidation. This indicated that fluid milk could be stabilized against this type of off-flavor development through proper selection and preservation of common forage.

Ozone was found to be the air pollutant responsible for the development of off-flavor in spray-dried milks produced in the Dairy Products Laboratory pilot plant during summer months. Seasonal changes in ozone concentration in Washington air correlate well with initial flavor changes in Dairy Products Laboratory spray-dried products.

2. Compounds Contributing to Flavor in Concentrated Milks. When milk is concentrated to double its normal solids content and autoclaved to achieve sterilization, the methyl ketone content of the product rises 30 to 60% above that of the unheated control. The significance of vanillin and maltol in the development of off-flavors in stored evaporated milks has been further investigated as well as the role of kynurenine and whey proteins as precursors for the development of o-aminoacetophenone in old dairy products.

In PL-480-supported research at the National Dairy Research Institute, Karnal, Punjab, India, it was found that the sulfhydryls and disulfides present in milk products made from both buffalo and cow milk result primarily from heat treatment encountered prior to analysis. Most of the sulfur compounds giving cooked flavors to the materials analyzed were proved to be dialyzable. The sulfhydryls all tended to oxidize on long-term storage. Phosphatase reactivation in heated milks was found to result from the sulfhydryls produced by the heat treatment.

3. Control of Flavor in Milk Fat. In contract research at Pennsylvania State University, radioactive isotope tracer techniques revealed that acetate is the common precursor of both the hydroxy and saturated acids produced in the secretory cells of the mammary gland. This indicates that genetically-controlled metabolic variations might exist which would lead to low lactone precursor production in milk fat. A positive correlation has been found between the lactone and methyl ketone producing capacity of milk fat. Therefore, if animals can be found that produce low levels of lactone precursor in their milk, products (especially whole milk powder) made therefrom should undergo little or no nonoxidative change during storage. This could lead to the elimination of "stale" flavor in dairy products stored in inert atmosphere.

In contract research at Oregon State University, a large taste panel has been used in conjunction with advanced chemical techniques (gas chromatography plus mass spectroscopy) to determine the contribution of various butter constituents to desirable flavor. Diacetyl, dimethylsulfide, methyl ketones, aldehydes, short-chain fatty acids and delta and gamma lactones are of major importance in producing butter flavor. As the quantitative relationships are established between the concentrations of these materials in butter and the hedonic response of consumers it should be possible by using additives or modifying production techniques to establish the most acceptable ratio of flavor constituents in the finished product. Several previously unreported compounds have been found in the volatile fraction of milk fat. These include a series of even numbered methyl ketones with up to 14 carbon atoms, and several hydrocarbons. Heating cream prior to churning results in the introduction of 5-methyl-2-furfuraldehyde, furfuraldehyde and dimethylsulfide into the finished butter.

Steam distillation conditions were established which allowed the removal of volatile material from stale butteroil samples without production of serious artifacts. The fractions when added back to fresh butteroil reproduced the typical "stale" flavor found in products stored for extended periods under nonoxidative conditions. The peak heights of the major volatile constituents in the steam distillate that could be separated by gas chromatography correlated well with the intensity of stale flavor observed in the samples by a panel of skilled judges.

4. New Methods in Chemistry of Flavor. The isolation and identification of those trace compounds responsible for the flavor of dairy products was expedited by the development of a new micromethod for the study of alcohols,

amines and thiol compounds. These materials are isolated as derivatives of pyruvic acid 2,6-dinitrophenylhydrazone. Gas, column and thin-layer chromatography has been used to separate and classify the derivatives. Using these techniques approximately 20 primary and secondary saturated alcohols and 30 carbonyl compounds have been isolated from Cheddar cheese and identified. The volatile fraction of Danish blue cheese contained approximately 10 alcohols. A comparison of cheese volatile components identifiable by straight gas chromatography versus this derivatizing technique indicates a much higher order of sensitivity for the latter leading to a more complete analysis. The development of a micromethod for the oxidation of vic-glycols, epoxides and α -ketals and vic-diketones gave data indicating the presence of some of these structures in butterfat.

C. Color, Texture, and Other Quality Factors

In order to determine the fate of polyphosphates added to concentrated milk before sterilization analytical procedures suitable for their determination have been developed. By using a combination of ion-exchange chromatography and thin-layer chromatography it is possible to achieve good separation of ortho-, pyro-, tripoly-, trimeta- and tetrametaphosphates. A calcium specific membrane electrode showed that 3:1 sterile milk concentrates containing hexametaphosphate contained 20-30% less free calcium ion than the controls. Addition of orthophosphate prior to sterilization gave similar effects. Whey utilization in sterile concentrated beverages was investigated and sweet whey, cream, stabilizers and flavoring materials were successfully combined and sterilized. Age thickening was controlled by addition of polyphosphate.

The gelation of milk by heat, orthophosphate, pyrophosphate and rennin apparently is accompanied by the release of peptides from the casein fraction. One of the peptides is the well characterized glycomacropeptide containing sialic acid that was the subject of pioneer rennin coagulation studies. This peptide contains no aromatic amino acids. However, all milk coagulation studied under this project proceeds with the additional release of peptides containing aromatic amino acids. This aromatic amino acid containing peptide fraction has been found to be heterogeneous with molecules differing in both amino acid and sugar content. The chemical composition of members of this group is undergoing further study and the kinetics of their release during milk gelation is being established.

In PL-480-supported research at "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, data has been obtained on the effect of temperature (0-80°C.) on:

- (a) The viscosity of four skim milks in the concentration range 8.5 - 28.5% TS and one whole milk at 11% TS.
- (b) The thermal expansion of the above milks over the same temperature range.

These studies provide basic information currently needed for the development of concentrated milks with better physical stability during storage.

D. Microbiology and Toxicology

Continued research on the relationship of dipicolinic acid to bacterial sporulation has shown that dipicolinic acid inhibition of sporulation is dependent upon the glucose content of the medium. Dipicolinic acid in the spore stabilizes glucose dehydrogenase against thermal denaturation.

This study will provide basic information needed for the development of sterile milks with less cooked flavor than those now available.

E. Technology - Process and Product Development

1. Dry Whole Milks. Foam stability in the drying process for vacuum dried milk is achieved by varying the temperature and rate of cooling of the concentrated milk as it passes through the chilling-foaming sector of the process. 8,000 cans, each reconstituting to one quart of fluid milk, were manufactured and test-marketed in cooperation with the ERS at a selling price 4 cents per quart less than fluid whole milk. Cost estimates show that the product can be sold profitably at this price with current raw material costs.

Foam-spray dried whole milk will be ready for market testing when suitable packaging devices become available through cooperation with American Can Co. The former seasonal flavor problem with this product was solved by passing dryer air through a carbon filter.

2. Skim Milk Powders. Skim milk powders having sinking and dispersing properties very similar to commercial "instantized" products can be made in the Dairy Products Laboratory spray dryer without further manipulation by injecting low levels of CO₂ into the concentrate immediately before atomizing, and operating the dryer under conditions to allow the powder to retain the maximum legally permissible levels of moisture. The mechanics of this in-dryer agglomeration is being investigated since its application to large commercial operations would be desirable. All skim milk powders have been found to undergo chemical change when held at dryer temperatures. A powder cooler has been designed which can rapidly drop the temperature of newly-made milk powders to safe levels. Agglomerated powders have been found very sensitive to mechanical vibration of the type encountered during transportation. The volume occupied by "instant" skim milk powder can be reduced as much as 35% by shaking. This makes directions for volume based reconstitution inaccurate. By controlling total solids content of concentrates, seeding and suitable temperature shifts prior to drying the crystalline lactose content of dehydrated dairy products can be drastically raised.

3. Heat Transfer in Powdered Milk. In contract research at Michigan State University, equipment allowing measurement of rate of heat transfer through beds of milk powder has been designed and tested. Raising bulk density and moisture content of similar products increases heat transfer rate. The influence of these transfer rates and cooling of powders in commercial bulk storage tanks and small packages has been started. Field studies of commercial powder cooling practices have begun.

4. Water Vapor Absorption by Dry Milk Products. In contract research at North Carolina State University, water vapor absorption by dehydrated dairy products and purified dry milk components has been measured at 24 C. Additional absorption studies at 35 C have been initiated to gather data relative to water binding energy involved in dehydrated dairy product reconstitution. The pattern of water absorption by milk powders indicates initial binding at low relative humidity by protein. As humidity increases, lactose glass becomes the principal water binding site. After conversion of lactose glass to the hydrate, moisture is released and at high relative humidities the milk salts absorb water rapidly leading to formation of saturated salt solutions capable of rendering casein insoluble. This mechanism explains milk powder solubility loss during rehydration of dense powders and accounts for excellent dispersibility of foam-dried products. Gas displacement techniques have been used to determine true density of dehydrated milk products as well as their relative porosities.

5. Effect of Steam Injection on Physical Changes in Milk. In grant research at the North Carolina State University, when steam was injected into milk to raise it to temperatures necessary to achieve sterilization, the steam condensation path length and the sound wave patterns produced by steam bubble collapse depended upon injector design. Small fluctuations in internal injector pressure were found to have little effect on downstream steam sound patterns. This indicates that careful control of pressure is necessary when attempting to evaluate steam injector performance on the basis of sound patterns produced by bubble collapse. Since much of the wave energy is produced in the ultrasonic region, uncontrolled pressure-induced sound pattern variation could lead to variability in change of physical structures in milk during steam injection.

6. Antioxidant Distribution in Fat-Containing Dairy Products. When gallic acid esters are added to a model system containing butter oil and water, partitioning occurs. The value of the partition coefficient depends upon the chain length of the gallate, temperature and the ionic strength of the aqueous phase. Partition of gallate antioxidants added to milk is complicated by the binding of the gallates by milk proteins. The extent of this binding is dependent upon chain length of the gallates showing about a two-fold increase for each additional CH_2 group in the side chain. Conditions derived from these model studies are being used to achieve most effective distribution in whole milk powders. Selective leaching of milk powder constituents with concentrated ammonium sulphate solutions demonstrates both a continuous lactose and protein phase in the powder particle. The location of fat globules in this multiphase matrix is undergoing study.

7. Anhydrous Milk Fat. The flavor and flavor stability of anhydrous milk fat have been improved by applying variations of the Indian ghee manufacturing technique to batch and continuous production of butter oil. The heat-treated butter oils so made have been tested for use in food manufacture. Promising results have been obtained for their use in candies and ice milks. They also add flavor to selected vegetables and seafoods. Fundamental data pertaining to the melting, solidification and crystallization of milk fat have been obtained using differential thermal analysis. This technique has been further used to study properties of fully and partially hydrogenated butter oils. Methods have been developed to hydrogenate butter oil to iodine values below 1. A large sample of this type of material has been sent to a chocolate candy manufacturer who will test the ability of this material to prevent the whitening of chocolate-coated candies during storage.

8. Cheese. Methods have been devised to produce a high quality natural skim milk cheese by proper control of bacterial starters, acid development, salt moisture ratios and pH change during manufacture and curing. Though the shelf life of the natural product is relatively short, it can be maintained in the frozen state for at least one year without quality loss. Processing the natural skim milk cheese improved its shelf life, but the flavor quality suffers in the judgment of skilled cheese tasters. The pilot plant studies have been carried out to the point where sufficient products can be manufactured for consumer market tests.

RPA 601 - EXPANSION OF MARKETS FOR U.S. PRODUCTS

A. Chemical Composition and Physical Properties (no current research)

B. Flavor (no current research)

C. Color, Texture and Other Quality Factors (no current research)

D. Microbiology and Toxicology (no current research)

E. Technology - Process and Product Development

In order to reduce environmental pollution by cheese wheys a mild, sweet, cereal flavored, nutritious powder suitable for reconstitution for beverage purposes was developed. This material can be made by pasteurizing, homogenizing, concentrating and spray drying mixtures of soy flour and liquid sweet whey. All operations can be carried out using conventional dairy plant equipment. Homogenization effectively improves the dispersion of soy flour solids and concentration and spray drying further reduce soy flavor partially masked by the whey solids. The material can be sweetened and flavored to meet consumer requirements. The protein efficiency ratio of the mix is equivalent to casein. A similar beverage can be made from cottage cheese whey and soy flour. Addition of citrus flavor, sugar, sucaryl and stabilizers yields a material having a consumer acceptance near that of fresh milk. Methods to reduce this material to a stable, soluble powder are being investigated.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND
NATURALLY OCCURRING TOXINS

- A. Chemical Composition, Physical Properties and Structure (no current research)
- B. Flavor (no current research)
- C. Color, Texture and Other Quality Factors

1. Milk Allergy. Available assay methods in animals for human milk allergens are not satisfactory. New information on antibody formation and passive and active systemic anaphylaxis in rabbits and rats is clarifying the difficulties in correlating reactions in humans with those in animals.

In tests with sera from 9 children who gave positive responses to milk by direct skin tests, only 2 of these contained milk reagins by passive transfer tests on prisoner volunteers. κ -Casein is not a major milk allergen in humans even though it is a strong sensitizing agent in animals.

Comparative studies of immunoglobulins isolated from cow's milk, colostrum, and blood serum using immunoelectrophoresis have demonstrated all preparations to be heterogeneous. Furthermore, the data so obtained tend to refute the common concept that immunoglobulins in the blood stream are transferred unchanged to milks. If such transfer does occur, it must favor the γ G-globulins. Moreover, the γ G- and γ A-globulin found in milk have electrophoretic properties different than those found in serum. Glycoprotein-a, a subunit of the apparent γ A-globulin of milk is not detectable in normal bovine serum and therefore may be synthesized in the mammary gland. The immunoglobulin fraction of milk is apparently complex showing possible effects of both genetic and physiological variations.

New antigenic specificities were found in pepsin hydrolyzates of partially purified milk protein fractions. Uterine strips taken from guinea pigs and sensitized with alum precipitates of the hydrolyzates indicated the presence of new specificities only in preparations obtained after 3 minutes of enzyme action at pH 3 and 4. Hydrolysis at lower or higher pH's yielded no active material, and extended hydrolysis times reduced potency of active preparations. The improved procedure of determining tryptophan has been successfully applied to the analysis of a variety of materials including corn meal, swine milk, "Red Tide" (Gonyaulax polyedra), γ -casein A, γ -casein B, cow lactalbumin and buffalo lactalbumin.

In PL-480-supported research at Hebrew University, Hadassah Medical School, Jerusalem, Israel, powdered milk was found to be more suitable for infants suffering from milk intolerance than pasteurized milk. Anti-milk antibodies in pregnant women or in newborn babies were very low or undetectable. An immune response to milk developed in almost all normal infants on a milk diet. The reaction starts a short time after the exposure to milk and reaches its peak 2 to 3 months later. These very thorough investigations of

the immune reactions of infants to milk should enable the dairy industry to appraise reports of milk sensitivity.

These studies provide basic information needed for the development of ways to eliminate or inactivate allergens in milk.

D. Microbiology and Toxicology

1. Salmonella in Milk Powders. Milk powders artificially contaminated with salmonella were used in studies of the effect of temperature, moisture, and chemical additives on the survival of these organisms. None of the food preservatives known to inhibit salmonella in liquids had any significant effect on survival in the dry state. The organism proved to be extremely stable to heat when moisture content of milk powders was maintained at commercially permissible levels. It became evident from this work that acceptable alterations in present powder manufacturing conditions offer little possibility of effective control. The study emphasizes the need for proper sanitation during concentrating, agglomerating and drying nonfat dry milk.

This study provides basic information showing that Salmonellae cannot be destroyed in the dry powder under practical conditions, and that sanitary precautions must be carefully observed to prevent contamination of the instantizing equipment in the manufacture of "instant" dry skim milk.

E. Technology - Process and Product Development

1. Storage Stability of Fluid Milk During Iodine-131 Decay. Contract research with Pet Milk Co. established that fluid milk can be processed and stored long enough for decay of contaminating radioactive Iodine-131 while retaining acceptable flavor and bacteriological quality. Pasteurization temperatures, storage temperatures and season of milk production were important factors in determining product shelf life. Re-pasteurization after the bacterial lag phase ended greatly extended storage life. Heating at 200 to 220°F. for 0.5 to 16 seconds allowed milk to maintain acceptability for 20 weeks when stored at 32°F. Lowering storage temperature from commonly used 45°F. to 32°F. increased shelf life of commercially produced summer milk five-fold. At all storage temperatures summer milk could be stored twice as long as winter milk. Milk spoilage during distribution could be markedly reduced by reducing temperature of storage cabinets.

RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

- A. Chemical Composition, Physical Properties and Structure (no current research)
- B. Flavor (no current research)
- C. Color, Texture and Other Quality Factors (no current research)

D. Microbiology and Toxicology (no current research)

E. Technology - Process and Product Development

1. Concentration and Fractionation of Dairy Products by Reverse Osmosis. In order to make whey disposal less costly and its utilization more economically attractive, research was undertaken to determine the effectiveness of reverse osmosis in the concentration of whey. Using commercially available reverse osmosis equipment, water removal rates could be increased significantly by utilizing elevated pressures and flow-through rates. These rates of water removal decreased as the solids concentrate of the feedstock increased. Preliminary results indicate that it is economically feasible to concentrate whey four-fold by using reverse osmosis pressures of 800 psi. Above this point further concentration requires pressures that make the operation uneconomical.

Publications and Patents - USDA and Cooperative Programs

RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

Chemical Composition and Physical Properties

- Alais, C., Kiger, N., and Jolles, P. 1967. Action of heat on cow κ -casein. Heat caseino-glycopeptide. J. Dairy Sci., 50, 1738-1743.
- Alais, C. and Julles, P. 1967. Isolation, purification, and analysis of two κ -casein-like fractions from sheep casein. J. Dairy Sci., 50, 1555-1561.
- Bingham, E. W., and Kalan, E. B. 1967. The ribonuclease B of bovine milk. Arch. Biochem. Biophys., 121, 317-324.
- Cerbulis, J. 1967. Distribution of lipids in various fractions of cow's milk. J. Agr. Food Chem. 15, 784-786.
- Cerbulis, J., and Ard, J. S. 1967. Method for isolation and detection of dioctyl phthalate from milk lipids. J. Assoc. Off. Anal. Chemists, 50, 646-650.
- Cerbulis, J., and Custer, J. H. 1967. Casein components soluble in chloroform-methanol (2:1) and in 50% aqueous ethanol. J. Dairy Sci., 50, 1356-1359.
- Downey, W. K., and Andrews, P. 1966. Studies on the properties of cow's milk tributyrinases and their interaction with milk proteins. Biochem. J., 101, 651-660.
- Ganguli, N. C., Gupta, S. K., Joshi, V. K. and Bhalerao, V. R. 1967. Sialic acid and hexose contents of proteose-peptone of milk in relation to other milk proteins. Indian J. Dairy Sci., 20, 96-102.
- Gorbunoff, M. J. 1967. Exposure of tyrosine residues in protein: Reaction of cyanuric fluoride with ribonuclease, α -lactalbumin and β -lactoglobulin. Biochem., 6, 1606-1615.
- Groves, M. L., and Gordon, W. G. 1967. Isolation of a new glycoprotein-a and a γ G-globulin from individual cow milks. Biochem., 6, 2388-2394.
- Homer, D. R., and Virtanen, A. I. 1968. Milk lipase (tributyrinase) in the milk of cows on protein-free feed. Milchwissenschaft, 23, 163-166.
- Noelken, M. 1967. The molecular weight of α_{s1} -casein B. Biochim. Biophys. Acta, 140, 537-539.
- Noelken, M., and Reibstein, M. 1968. The conformation of β -casein B. Arch. Biochem. Biophys., 123, 397-402.

- Noelken, M. E., and Timasheff, S. N. 1967. Preferential solvation of bovine serum albumin in aqueous guanidine hydrochloride, *J. Biol. Chem.* 242, 5080-5085.
- Patton, S., Chandler, P. T., Kalan, E. B., Loeblich, A. R. III, Fuller, G., and Benson, A. A. 1967. Food value of red tide (*Gonyaulax polyedra*). *Science*, 158, 789-790.
- Phillips, Nancy I., Jenness, Robert, and Kalan, Edwin B. 1968. Immunochemical comparison of β -lactoglobulins. *J. Immunol.*, 100, 307-313.
- Phillips, N. I., Jenness, R., and Kalan, E. B. 1967. Reactivity of sulfhydryls in several β -lactoglobulins. *Arch. Biochem. Biophys.*, 120, 192-197.
- Schwartz, Daniel P. and Virtanen, Artturi, I. 1967. Normalcy of some non-volatile carbonyl compounds and carbonyl precursors in the fat of synthetically-fed cows. *Acta Chem. Scand.*, 21, 2583-2584.
- Seibles, T. S., and Weil, L. 1967. Reduction and S-alkylation with acrylonitrile. Pub. In "Methods in Enzymology." Edited by S. P. Colowick and N. O. Kaplan. New York, Academic Press. Vol. 11, 204-206.
- Susi, H., Timasheff, S. N., and Stevens, L. 1967. Infrared spectra and protein conformations in aqueous solutions. I. The amide I band in H_2O and D_2O solutions. *J. Biol. Chem.*, 242, 5460-5466.
- Syvaoja, E. and Virtanen, A. I. 1968. Studies of enzymes in milk produced with normal feed and protein-free feed. *Milchwissenschaft*, 23, 200-204.
- Thompson, M. P., Kalan, E. B., and Greenberg, R. 1967. Properties of caseins modified by treatment with carboxypeptidase A. *J. Dairy Sci.*, 50, 767-769.
- Timasheff, S. N., Susi, H., and Stevens, L. 1967. Infrared spectra and protein conformations in aqueous solutions. II. Survey of globular proteins. *J. Biol. Chem.*, 242, 5467-5473.
- Timasheff, S. N., Susi, H., Townend, R., Stevens, L., Gorbunoff, M. J., and Kumosinski, T. F. 1967. Application of circular dichroism and infrared spectroscopy to the conformation of proteins in solution. In "Symposium on Conformation of Biopolymers," G. N. Ramachandran, ed. N.Y., Academic, Vol. 1, 173-196.
- Townend, R., Kumosinski, T. F., and Timasheff, S. N. 1967. The circular dichroism of variants of β -lactoglobulin. *J. Biol. Chem.*, 242, 4538-4545.
- Virtanen, A. I. 1967. New view in cattle feeding; normal concentrates replaced by urea and hemicellulose syrup prepared from wood. *Agrochimica*, 11, 289-321.

Virtanen, Artturi I. 1967. Milk production on a protein-free feed, using urea and ammonium salts as the sole source of nitrogen. *Anales Edafol. Agrobiol.*, 26, 447-468.

Zittle, C. A. 1967. Precipitation of caprine and bovine caseins from acidic solutions by sodium polyphosphate: Influence of pH and area. Utilization for separation of α_s - and κ -caseins. *J. Dairy Sci.*, 50, 1352-1355.

Flavor

Dimick, P. S., and Harner, J. L. 1968. Effect of environmental factors on lactone potential in bovine milk fat. *J. Dairy Sci.*, 51, 22-27.

Schwartz, D. P., and Brewington, C. R. 1967. Methods for the isolation and characterization of constituents of natural products. V. Separation of 2,6-dinitrophenylhydrazone pyruvamides into classes and resolution of the individual members. *Microchem. J.*, 12, 547-554.

Schwartz, D. P., and Brewington, C. R. 1967. Methods for the isolation and characterization of constituents of natural products. IV. Amide derivatives of amines with pyruvyl chloride 2,6-dinitrophenylhydrazone. *Microchem. J.*, 12, 192-195.

Schwartz, D. P., Brewington, C. R., and Shamey, Jennie. 1967. Methods for the isolation and characterization of constituents of natural products. III. Separation of alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazone into classes by column and thin-layer chromatography. *Microchem. J.*, 12, 186-191.

Schwartz, Daniel P. and Virtanen, Artturi I. 1967. Normalcy of some non-volatile carbonyl compounds and carbonyl precursors in the fat of synthetically-fed cows. *Acta Chem. Scand.*, 21, 2583-2584.

Color, Texture and Other Quality Factors

Schormüller, J., Grampp, E., and Belitz, H. D. 1968. Veränderungen von milchproteinen durch einwirkung von carbonylverbindungen. I. Mitteilung. Reaktion von casein mit athanal. *Z. Lebensm.-Untersuch. Forsch.*, 136, 271-279.

Microbiology and Toxicology

Ben-Ishai, R., and Zeevi, N. 1967. Postirradiation cell division in 5-fluorouracil-pretreated *Escherichia coli*. 1967. *J. Bacteriol.*, 93, 749-753.

Technology - Process and Product Development

Aceto, N. C., Craig, J. C., Jr., Eskew, R. K. and Talley, F. B. 1966. Storage aspects of continuous vacuum foam-dried whole milk. 17th

International Dairy Congress Proceedings, Munich, Section E:3, 189-196.

- Arbuckle, W. S., Blanton, L. F., Walter, H. E., and Sadler, A. M. 1967.
Using heat-treated milk fat in frozen dairy foods. Ice Cream Field
Ice Cream Trade J., 149, 38, 40, 46.
- Berlin, E., Lakshmanan, S., Kliman, P. G., and Pallansch, M. J. 1967.
Isolation and physicochemical characterization of a lipoprotein fraction
from bovine milk. Biochem., 6, 1388-1394.
- DellaMonica, Edward S., Craig, James C., Jr. and Calhoun, M. J. 1968.
Error in the analysis of hydroxymethylfurfural in processed milk. J.
Dairy Sci., 51, 352-355.
- DellaMonica, Edward S., Holden, T. F. 1968. Comparison of toluene distilla-
tion and Karl Fischer methods for determining moisture in dry whole milk.
J. Dairy Sci., 51, 40-43.
- Fox, K. K., Holsinger, V. H., Posati, L. P., and Pallansch, M. J. 1967.
Composition of granules in evaporated milks stored at low temperatures. J.
Dairy Sci., 50, 1032-1037.
- Fox, K. K., Holsinger, V. H., Posati, L. P., and Pallansch, M. J. 1967.
Separation of β -lactoglobulin from other milk serum proteins by trichloro-
acetic acid. J. Dairy Sci., 50, 1363-1367.
- Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1967. Denaturation of
cottage cheese whey proteins by heat. J. Dairy Sci., 50, 828-832.
- Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1967. Use of cottage cheese
whey solids in sponge bread. Bakers Dig., 41 44-46, 48-50.
- Hanrahan, F. P., Selman, R. L., and Webb, B. H. 1967. Experimental equip-
ment for cooling and packaging foam-spray-dried milk in the absence of
oxygen. J. Dairy Sci., 50, 1873-1877.
- Holsinger, V. H., Posati, L. P., and Pallansch, M. J. 1967. Rapid determi-
nation of chloride concentration of cheese by use of a Pungor electrode.
J. Dairy Sci., 50, 1189-1193.
- Kliman, P. G., and Pallansch, M. J. 1967. Attenuated total reflectance of
infrared energy by dairy products. J. Dairy Sci., 50, 1211-1215.
- Kurtz, F. E. 1967. Possible inclusion of artifacts in flavors recovered
directly from dried whole milk. J. Dairy Sci., 50, 814-817.
- Srinivasan, R. A., Chakravorty, S. C., Babbar, I. J., and Dudani, A. T. 1967.
Proteinase system of a fusarium culture. Indian J. Dairy Sci., 20, 86-88.

Tamsma, A., Kontson, A., and Pallansch, M. J. 1967. Influence of drying techniques on some properties of nonfat dried milk. J. Dairy Sci., 50, 1055-1060.

Tamsma, A., Kurtz, F. E., and Pallansch, M. J. 1967. Effect of oxygen removal technique on flavor stability of low-heat foam-spray-dried whole milk. J. Dairy Sci., 50, 1562-1565.

Tamsma, A., Kurtz, F. E., Berlin, E., and Pallansch, M. J. 1967. Flavor of recombined milk. J. Dairy Sci., 50, 1878-1881.

Yoncoskie, R. A. 1967. Calorimetric studies on the heat of melting of milk fat by differential thermal analysis. J. Am. Oil Chemists' Soc., 44, 446-448.

RPA - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

Color, Texture and Other Quality Factors

Spies, J. R. 1967. Determination of tryptophan. Anal. Chem., 39, 1412-1416.

Microbiology and Toxicology

McDonough, F. E., Hargrove, R. E., and Tittsler, R. P. 1967. The fate of Salmonellae in the manufacture of cottage cheese. J. Milk Food Tech., 30, 354-356.

RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

Technology - Process and Product Development

McDonough, F. E. 1968. Whey concentration by reverse osmosis. Food Eng. 40, No. 3, 124-127.

MEAT UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y. 1968		
	: Research Problem Area	:	: Total
	: 410	: 702	:
Pennsylvania (Wyndmoor)	:	:	:
Maryland (Beltsville)	:	:	:
	:	:	:
A. Chemical Composition and Physical Properties	: 3.0	: 0	: 3.0
B. Flavor	: 4.0	: 0	: 4.0
C. Color, Texture and Other Quality Factors	: 5.0	: 0	: 5.0
D. Microbiology and Toxicology	: 4.2	: 1.4	: 5.6
E. Technology - Process and Product Development	: 1.4	: 0	: 1.4
Total	17.6	1.4	19.0

Intramural program is supplemented by extramural support representing (a) 4.6 SMY's at universities and at state agricultural experiment stations^{1/}, (b) 0 SMY's at other U.S. institutions, and (c) PL-480 funds in 7 countries representing 412,196 U. S. dollars equivalent.

^{1/} RPA 410 - Color, Texture and Other Quality Factors, 1.2; Technology - Process and Product Development, 2.9;

RPA 702 - Microbiology and Toxicology, 0.5.

Problems and Objectives

Shelf life of fresh meat, currently about 5 days, needs to be lengthened so that more efficient processing and packaging in centralized locations with resultant economies is possible. Processed meat products, two-thirds of our pork and significant quantities of other meats, need better keeping quality relative to color and flavor retention in distribution channels. Technical advances are needed to enable the numerous small meat processors to manufacture better products. Contamination of meat with pathogenic microorganisms is a serious problem which must be eliminated through improved processing techniques.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Prolong the retail shelf life of fresh meat to 15 to 20 days.
2. Provide procedures for the preparation of meat products with initially desirable organoleptic and physical characteristics which are retained in storage.
3. Provide technology which can be applied to the problems of small-scale processors and improve the meat products of rural industry.
4. To improve meat processing methods so that the consumer can be assured of safety in meat and its products.

Progress - USDA and Cooperative Programs

RPA 410 - NEW AND IMPROVED MEAT AND MILK PRODUCTS

A. Chemical Composition and Physical Properties

Comparison of enzymic activity of myosin from beef, pig and rabbit muscles was continued. Kinetic studies of enzymic activity revealed that Michaelis constants and maximal hydrolysis velocity values were similar for beef and pig myosins, with differences for rabbit myosins that were statistically affirmed. Myosin acts both as a structural protein important in muscle contraction, and as an enzyme which splits adenosine triphosphate (ATP). The present studies on the differences between the ATP-ase activity of the myosin of different muscles may explain the differences in the tenderness, water-holding capacity, and succulence of meat from different livestock sources.

In PL-480-supported research at the University of Helsinki, Finland, determination of organic acids in ripened dry sausage showed that in addition to lactic acid volatile fatty acids are also formed during ripening. Chief of these is acetic acid with an appreciable amount of formic acid. The concentration of alcohols and lactones is much less than that of carbonyl compounds, 1 or 2 of which could account for the flavor fault in sausage

innoculated with lactobacilli. This may be prevented by using a mixed culture of micrococci and lactobacilli.

These studies provide basic information needed for the development of improved meat products.

B. Flavor

1. Meat. Water-soluble extracts of beef yield a roast aroma on pyrolysis. The precursors of the aroma are present in the nonprotein, low molecular weight fraction. The nucleic acid derivatives, such as inosinic acid, inosine and hypoxanthine, which are possibly implicated in flavor, can be removed from the extract without seriously affecting development of roast aroma on pyrolysis. Combining fractions containing sugars and amino acids, none of them smelling particularly meaty on pyrolysis, resulted in the development of a meaty aroma. The role of fat in the flavor of meat was studied with taste panel evaluations. Beef fat probably plays a minor role in the development of the characteristic aroma in pyrolyzed beef. The flavor precursors in pork fat are water-soluble and may be removed by appropriate treatment. Lamb fat also contributes to the lamb odor; however, the precursors are not water-soluble and are concentrated in the fat by the procedure used.

In grant research at Rutgers University the volatile flavor compounds from cooked beef were separated into neutral and acidic fractions. The neutral fraction was further separated into 18 portions, some of which had onion, garlic, butter and meat odors. Individual compounds are being identified by infrared and mass spectrometry. Some of the carbonyl compounds contain cyclic rings. The volatile compounds formed by cooking fresh beef in water will serve as a control for study of compounds formed by rancid beef.

2. Smoke. The thermal degradation of ferulic acid, a possible breakdown product of lignin, produced several compounds found in wood smoke, indicating that the mechanism of their formation from lignin is through ferulic acid. In identification studies of smoke components, butyrolactone and levulinic acid, not previously reported, were found in a commercial liquid smoke preparation. 2-Methyl-2-cyclopenten-1-one, also previously unreported in smoke, was identified in a smoke condensate prepared in this laboratory. A taste panel found a simple mixture of synthetic constituents to be as smokey as, or smokier than, the original commercial smoke preparation. The synthesis of a smoke solution from known chemicals, based on information obtained by fractionation, will lead to a simpler, more economical and safer smoked meat production.

These basic studies on meat flavor are currently needed for the development of better meat products.

C. Color, Texture and Other Quality Factors

A model system for study of factors involved in the lipid autoxidation of meat consisted of stable gels made from sodium carboxy methyl cellulose, lard, and water. The effect of additives on autoxidation characteristics of this mixture was evaluated for frozen, freeze-dried, or untreated systems. When freeze-dried, a sponge-like structure was obtained which oxidized with great rapidity. When well aerated in preparation, the gels autoxidized at a satisfactory rate at 20°C. in the dark. In preliminary experiments, sodium chloride, myoglobin, cytochrome C, and EDTA were used as additives. Sodium chloride had an independent pro-oxidant effect. EDTA in the presence of tocopherol and the heme compounds had a marked antioxidant effect. To date it is not clear that this is due to metal chelation.

Although a number of green heme pigments are known, the identification of any of them as the discoloration of cured or fresh meats has been accomplished in only one or two cases. Under the conditions that occur in meat, the pigments are relatively insoluble and not amenable to identification, and they usually occur as transient intermediates and readily decompose. Identification studies have begun with the development of techniques for cleaving the heme-protein bonds, and of solvent systems in which the cleaved hemes would be soluble. A novel green heme-protein pigment with unique optical absorption maximum at 589 nm has been observed from the action of hydrogen peroxide on metmyoglobin at pH 4.5. A cell has been constructed for the study of cured meat color changes in frankfurter emulsions. The cell is constructed to allow for stepwise adjustment of pH or e.m.f. electrodes, and so designed as to eliminate or reduce the effects of stray light. It will be used for studies of the kinetics, thermodynamics, and pH and concentration dependence on the formation of the nitrosylheme pigments of cured meats.

In PL-480-supported research at Technical University, Gdansk, Poland, antioxidant properties of curing smokes were investigated, and those compounds having both phenolic and carbonyl or carboxyl groups were found to have the greatest antioxidant properties. A two-stage smoke generation system was developed in which heating and oxidizing of the wood were separated. This gave better flavor, better antioxidant qualities, and was more controllable than conventional smoke generators.

In PL-480-supported research at the British Food Industries Research Association, Leatherhead, Surrey, England, very low levels of oxygen (0.1%) may interfere with the conversion of myoglobin to nitrosylmyoglobin. The addition of ascorbate in amounts ranging from 90 to 900 ppm was effective in preventing oxygen interference with nitrosylmyoglobin formation.

These studies provide basic information needed for the development and of improved meat products.

D. Microbiology and Toxicology

Autolysis of isolated cell walls of a psychrophilic Bacillus sp. has been shown to be inhibited by yeast extract or peptone but not by glucose or casamino acids. This inhibition of autolysis suggests that this psychrophilic bacterium has a unique metabolic control mechanism. The effect of several additional microbial species on the development of peroxides and monocarbonyls in fresh lard has been studied. Most cultures prevented the development of peroxides; Staphylococcus aureus prevented the development of monocarbonyls as well. Pseudomonas fragi and Candidum lipolytica produced a marked increase in alkanals and methyl ketones and Geotrichum candidum in alkanals. The activity of microorganisms in reducing carbonyl content of fats has an important bearing on meat product flavor development.

Contract research at Iowa State University is now completed and the following points of significance were shown: (1) Molds are not required for typical flavor development in cured hams and fermented sausages, but may contribute indirectly to flavor by limiting rancidity development; (2) fat of those hams that had no mold growth was more yellow, translucent, softer, and more rancid; (3) Penicillium expansum was the most common mold isolated during the first six months of aging and Aspergillus ruper and A. repens after six months; (4) salt is the only curing agent required, but nitrite, sugar, and smoke can contribute to color and flavor; (5) molds are commonly associated with Hungarian and California-style salami; (6) adequate knowledge is now available to produce country-cured hams or dry sausages of good quality in any geographical area which can duplicate special products of local significance.

In PL-480-supported research at the University of Veterinary Medicine, Vienna, Austria, the heat resistance of streptococci was found to be significantly reduced by the addition of lauryl gallate, phyophosphates, metaphosphates, and lysozyme. Mixtures of some of these substances were more effective than single compounds. Extracts of erythrocytes had similar effects. Addition of sodium caseinate improved the heat conductivity of meat-fat emulsions.

These basic microbiological studies are needed to aid in the improvement of fresh, cured and cultured meat products.

E. Technology - Process and Product Development

1. Emulsion-type Products. In the preparation of meat emulsions, an explanation was sought for the observation that high-melting fats, although melted during emulsification, tend to yield stable emulsions, while the same amount of liquid fat does not. Experiments with oily fat showed that emulsion stability was markedly extended if the fat was added in portions to lean meat batters, rather than as one addition as is the general practice in meat emulsification. The results suggest that melting of fat during emulsification meters the rate at which oily fat becomes available and thus produces a stable emulsion. A study of means of stabilizing unstable emulsions

showed that chilling and storing, or chilling and a second comminution, were effective with some emulsions, using stability on cooking as a criterion. Preliminary results show that changes in viscosity accompany, and possibly anticipate, onset of emulsion instability.

Contract research continued at Michigan State University on the development of new smoked meat products. The use of smoked nonfat dry milk to introduce smoked flavor into emulsion-type meat products was investigated with emphasis on determining the level of smoking the dried milk should receive. Progress was made in the development of two other new products, a smoked corned beef product and a product from lamb breasts. Curing and smoking lamb riblets improved their acceptability to taste panelists. Statistical analysis of data obtained using a 150-member consumer panel confirmed a conclusion given in a previous report that content of phenol bears the closest relation to smoke perception of any individual additive.

2. Thermal Treatment Indicators. Cooking experiments were conducted with canned hams to systematically determine the accuracy of residual enzyme content as an indication of cooking temperature. Results indicate that, within the range of commercial practice, the temperatures attained when canned hams are heat-processed can be estimated to within at least $\pm 6.1^{\circ}\text{F}$. by determination of content of acid phosphatase.

3. Cooked Meats. Experiments with pork spare ribs, beef briskets, and chickens (broilers) indicated that the microwave barbecuing method produced a comparable product in one-half the time of the conventional barbecuing method. There was no significant difference in the moisture content and Kjeldahl-nitrogen of meat samples barbecued by the two methods. The finished product from the microwave method contained 5-10% less crude fat. This contract research at Southern University is aimed at developing ready-to-eat products which can be produced in small plants in rural communities.

In contract research at Cornell University, the composition of 60 oven-prepared rib roasts was determined in terms of percentage of outside fat covering, seam fat, fat inside ribs, separable lean, inseparable lean and fat-boned and tendon. The results indicated that left and right sides are similar except for small differences in fat inside ribs which can be equalized by trimming. Based on this, one of a pair can be used for cookery and the other for chemical and physical analysis in future work. Instruments for measurement of thermal properties have been successfully applied. In developing a method for following heat-induced changes in the proteins of meats during cooking, effective procedures were established for preparing and fractionating extracts. The data obtained from this research can be used to improve large-scale meat cookery and to improve equipment design.

RPA 702 - IMPROVED TECHNOLOGY TO PROTECT PEOPLE FROM TOXIC RESIDUES, MICROORGANISMS, PARASITES AND OTHER HAZARDS

A. Chemical Composition and Physical Properties (no current research)

B. Flavor (no current research)

C. Color, Texture and Other Quality Factors (no current research)

D. Microbiology and Toxicology

1. Growth of Staphylococcus aureus in Meats. In studies of salt-tolerant bacteria isolated from cured hams it was found that high salt concentrations in the medium would in some instances influence the results of some biochemical tests and thus can lead to incorrect identification of microorganisms. Production of enterotoxin B by a food poisoning strain of Staphylococcus aureus was shown to be more rapidly inhibited by increasing concentrations of NaCl than was cell growth. Low temperatures also apparently reduced the amount of toxin produced per cell. This study indicates, however, that commercial meat-curing practices afford little or no protection against enterotoxin B production by staphylococci.

2. Growth of Salmonellae in Meat. Fundamental information on the growth and survival of various strains of Salmonellae in culture media indicate a tolerance for salt and other curing agents. In pork containing 2% salt growth at 10° was normal.

3. Growth of Clostridium botulinum in Meats. In contract research at the University of California (Davis) the relationships for salt and pH in controlling the growth of Clostridium botulinum in meats are being investigated. Preliminary experiments to study the antagonistic effects of Pediococcus cerevisiae for three Clostridium botulinum types led to the interesting finding that radiation-killed cells of P. cerevisiae could substantially reduce the pH of meats if glucose was present.

E. Technology - Process and Product Development (no current research)

Publications and Patents - USDA and Cooperative Programs

RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

Flavor

- Ayres, J. C., Lillard, D. A., and Leistner, L. 1967. Mold-ripened meat products. Proc. 20th Reciprocal Meat Conference, 156-168.
- Fiddler, W., Parker, W. E., Wasserman, A. E., and Doerr, R. C. 1967. Thermal decomposition of ferulic acid. J. Agr. Food Chem., 15, 757-761.
- Leistner, L., and Ayres, J. C. 1968. Molds and meats. Fleischwirtschaft, 48, 62-65.
- Satterlee, L. D., and Lillard, D. A. 1967. A procedure for gas chromatographic analysis of free amino acids in meats. J. Food Sci., 32, 682-685.
- Wasserman, A. E. 1967. The chemistry of cooked meat flavor. Proc. 20th Reciprocal Meat Conference, 228-240.
- Zaika, L. L., Wasserman, A. E., Monk, C. A., Jr., and Salay, J. 1968. Meat flavor. II. Procedures for the separation of water-soluble beef aroma precursors. J. Food Sci., 33, 53-58.

Color, Texture and Other Quality Factors

- Hutchins, B. K., Liu, T. H. P., and Watts, B. M. 1967. Effect of additives and refrigeration on reducing activity, metmyoglobin and malonaldehyde of raw ground beef. J. Food Sci., 32, 214-217.
- Walters, C. L., Casselden, R. J., and Taylor, A. McM. 1967. Nitrite metabolism by skeletal muscle mitochondria in relation to haem pigments. Biochim. Biophys. Acta, 143, 310-318.
- Zalewski, S., and Gaddis, A. M. 1967. Effect of transesterification of lard on stability, antioxidant-synergist efficiency, and rancidity development. J. Am. Oil Chemists' Soc., 44, 576-580.

Microbiology and Toxicology

- Alford, J. A., and Steinle, E. E. 1967. A double-layered plate method for the detection of microbial lipolysis. J. Appl. Bacteriol., 30, 488-494.
- Mencher, J. R., and Alford, J. A. 1967. Purification and characterization of the lipase of Pseudomonas fragi. J. Gen. Microbiol., 48, 317-328.
- Nurmi, Esko. 1966. Effect of bacterial inoculations on characteristics and microbial flora of dry sausage. 1966. Acta Agral. Fennica, No. 108, Helsinki.

Smith, J. L., and Alford, J. A. 1968. The action of microorganisms on the peroxides and carbonyls of rancid fat. J. Food Sci., 33, 93-97.

Technology - Process and Product Development

Anonymous. 1967. Maximum emulsion chopping temperatures. Western Meat Ind., 13, 20, 34.

Townsend, W. E., Witnauer, L. P., Riloff, J. A., and Swift, C. E. 1968. Comminuted meat emulsions: differential thermal analysis of fat transitions. Food Technol., 22, 319-323.

RPA 702 - IMPROVED TECHNOLOGY TO PROTECT PEOPLE FROM
TOXIC RESIDUES, MICROORGANISMS, PARASITES
AND OTHER HAZARDS

Microbiology and Toxicology

Lilly, H. D. 1967. Effects of curing salts and temperature on production of staphylococcal enterotoxin B. M.S. Thesis, N. C. State Univ.

ANIMAL FATS AND OILS UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968	
	Research Problem Area	
	411	Total
Pennsylvania (Wyndmoor)		
A. Chemical Composition,		
Physical Properties and		
Structure	7.8	7.8
B. Chemical and Physical		
Investigations to Improve		
Products	27.3	27.3
Total	35.1	35.1

Intramural program is supplemented by extramural support representing (a) 1.8 SMY's at universities and at state agricultural experiment stations^{1/}, (b) 1.7 SMY's at other U.S. institutions^{1/}, and (c) PL-480 funds in 3 countries representing 183,000 U.S. dollars equivalent.

^{1/} RPA 411 - Chemical Composition, Physical Properties and Structure, 1.3; Chemical and Physical Investigations to Improve Products, 2.2.

Problems and Objectives

The livestock industry produces about 4-1/2 billion pounds a year of inedible animal fats. While production has doubled in the last 15 years, the principal outlet for inedible fat (in soap) has declined sharply. Utilization research has played a leading role in finding new uses for 1 billion pounds of this fat, the largest use being in animal feeds.

Additional uses are needed to provide more remunerative outlets for inedible animal fats. Emphasis is on realizing the potential of fats as a raw material for the chemical industry.

Objectives of the research are to develop and evaluate alternative procedures for:

1. Improving solubility characteristics of biodegradable detergents and surface-active agents based on animal fats.
2. Preparing fat-based urethane foams and coatings.
3. Synthesizing lubricant components and lubricant additives, particularly fat-derivatives of high thermal stability.
4. Developing fatty derivatives capable of crosslinking or stabilizing additive or condensation polymers.
5. Providing fat-based monomers for internal plasticizing of polymers.

Progress - USDA and Cooperative Programs

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

A. Chemical Composition, Physical Properties and Structure

In contract research at Villanova University, Villanova, Pa., further attempts were made to find a method for theoretically reproducing the experimental rotational barriers of propane and butane. The current set of computations is based on an electrostatic model in which the Slater exponent and charge for each atom are considered as adjustable parameters. The charge per atom is not a whole number and is allowed to change with angle of rotation about the bond. It was found that very small changes in the electron population, with angle of rotation, produce large changes in barrier energy. All geminal interactions are also included, leading to a substantial improvement in results. Using this model, the computations resulted in barrier energies in reasonable agreement with experimental data for ethane, propane, and butane. These computational experiments represent exploratory steps along the road toward theoretical understanding of the barrier to internal rotation in triglycerides.

Some of the molecular orbital computer programs have been obtained from Villanova University and have been revised to operate with the EU computer, making use of the capabilities of its disk storage. The large amount of

space available on a disk means that it will be possible to expand these programs to accommodate molecules of even larger size than is possible on Villanova's computer.

A new computer program for simulating countercurrent distribution (CCD) was developed at EU. This allows for variable partition coefficients and can use a real ternary diagram. It also takes into account solute-solvent interactions, solute volumes, mutual solubilities of solvents, mass reorganization and isopycnic points. Nonideal thermodynamic phenomena met in CCD can then be tested. With this program, it is possible to predict an output profile for a single solute in any two-phase system. This allows a closer approach to the real systems encountered in the physical chemistry of fats and other commodities. Insight into the plate theory of chromatography has also been obtained through these calculations.

A mathematical study was done on the effects of L-ethionine on transport of D- and L-methionine through a biological membrane. It was found that the equation for competitive inhibition at the steady state was obeyed throughout the concentration range studied. No evidence for enhancement could be found. This question could not be answered by simple examination of the experimental data but required the mathematical analysis.

A short lecture course was given to approximately 80 members of EU to acquaint them with our computer and how to use it. This has given them a clearer concept of how the computer could be helpful to their research projects.

In grant research at the University of Connecticut, Storrs, procedures have been developed for the large-scale preparation of glycerides, and several racemic and cryptoactive glycerides of very high purity have been prepared. A method has been developed to distinguish racemic pairs of glycerides containing oleic acid. An effective antioxidant (butyl hydroquinone) has been found to prevent oxidation of the highly unsaturated glycerides during synthesis.

In research at Technical University, Gdansk, Poland, under a PL-480 grant, a study of the kinetics and thermodynamics of methyl linoleate at three temperatures (60, 80 and 100°C.) in absence and presence of three concentrations of the antioxidant, propylgallate, has shown that the process is first order both in relation to the reaction substrate and an undefined catalyst which also may be formed in the first autoxidation step. A three-step kinetic scheme is proposed in going from unoxidized ester to peroxide decomposition products. This study indicates that the effect of the antioxidant is primarily to reduce active concentration of the initial catalyst, thus extending the induction period. An increase in temperature is antagonistic in relation to the antioxidant, increasing the catalyst concentration. After the induction period, the antioxidant has no distinct influence on reaction rate constants, free energy, entropy or enthalpy in activated complex formation.

A differential scanning calorimeter was calibrated with metals and organic compounds. Often-used data for the heats of fusion of some lipids have been shown to be incorrect. The tool has been shown to be useful for the detection of cryptoactive optical isomerism and in the prediction of conditions necessary for the purification of mixtures by zone refining.

High precision measurements have been carried out to evaluate the dielectric properties of long-chain esters related to fats. Complete experimental data have been obtained for pentadecyl tetradecanoate. A computer program was written to fit the data by polynomial equations. The equations are applied to calculate the dielectric constant and dielectric loss of the esters.

Polyunsaturated fatty acids with specific cis-trans isomerism were prepared. Santalbic acid can be converted to the corresponding cis-trans acid in 90-93% yield. The hydrogenation of 7,9-diynoic acid leads to cis-cis dienoic acid.

In research at Technical University, Gdansk, Poland, several new thermally stable, liquid stationary phases for gas-liquid chromatography have been synthesized and are in various stages of evaluation. One, isobutoxypolysiloxane, has a thermostability about 10% greater than silicone SE-30 which is one of the better high temperature liquid phases. A few studies have been made on the addition of stabilizing compounds to polyester liquid phases. Phenylneopentyl phosphite and orthophosphoric acid are most effective, depending on the particular polyester. An increase of 20 to 35% in thermostability was indicated with no observable change in the selectivity of the liquid phase for the separation of fatty acids.

These studies provide basic information needed for preparation and evaluation of derivatives from animal fats.

B. Chemical and Physical Investigation to Improve Products

1. Plastics Investigations. Fire-resistant (self-extinguishing) urethane foams were obtained using hypohalogenated glycerides as the polyol ingredient. Physical properties of the foams are comparable to those obtained from fat-based polyols containing no halogen.

Although allylstearamide forms only low polymers, the wax compositions resulting from treatment with benzoyl peroxide show promise for polishing applications. As a result of the extraordinary low glass transitions of their homopolymers, it is concluded that typical fatty comonomers are on a mole basis the most effective comonomers possible in this aspect of internal plasticization.

Solvents are shown to exert a strong effect on the mode of thiocyanogen addition to double bonds to give at least two types of compounds, vicinal dithiocyanate and vicinal isothiocyanate derivatives.

Double bonds were epoxidized by the new strong peracids pentafluoroperbenzoic and 3,5-dinitroperbenzoic acids in tetrachloroethane solvent 5000 times faster than by peracetic acid in methyl acetate. A new mild synthesis of peracids was achieved by perhydrolysis of the mixed anhydride, diethyl phosphoric acyl anhydride; this will permit synthesis of unusual peracids unobtainable by any other method.

A new synthesis of long-chain iodides consists in reacting benzoyl peroxide or t-butylperoxy isopropyl carbonate with a fatty acid and iodine.

Pure 10,11-methylene undecanoate was synthesized for a continued study of the mechanism of the acid catalyzed isomerization of unsaturated fatty acids.

Isopropenyl stearate, a versatile and potent new acylating agent, forms hexadecylketene, which self-condenses to new cyclic derivatives. The ester forms thiostearic anhydride from thioacetic acid, thioesters from mercaptans, amides from amines, imides from amides, and provides a new synthesis of stearyl chloride and fluoride.

The decrease in melting point and in apparent heat of fusion caused by crystalline octadecyl side chains was followed in a series of copolymers in which the other comonomers had amorphous side chains of varied lengths. There was evidence that crystalline size and perfection can be reduced by use of comonomer mixtures having both amorphous and crystalline side chains. Superior internal plasticizing might result from the use of fatty comonomers prepared from naturally occurring fat mixtures. Of special significance was the finding that 18-carbon oleyl side chains are non-crystalline down to -73°C . Thus, the undesirable properties sometimes conferred by side chain crystallinity can be entirely avoided using fat-derived oleyl comonomers.

2. Lubricant Investigations. The synthetic reaction by which an alkyl branch is introduced adjacent to the carboxyl group of fatty acids, thus giving these acids and their esters decreased volatility and increased hydrolytic stability, has been extended. The free-radical-initiated addition of these fatty compounds to aliphatic terminal olefins has been applied successfully to acyl halides (stearyl chloride), acyl anhydrides (stearic anhydride) and acid amides (stearamide) in their addition to 1-decene. The success with acyl halides is particularly significant, since the resulting alpha-branched acyl halides can be converted directly and easily to esters which cannot be prepared readily by any other method. The products are of interest as potential lubricant components.

The cyanoethylation of hydroxy fatty acids or esters with acrylonitrile introduces a new functional group, the β -cyanoethyl ether function, into the hydrocarbon chain of fats. The nitrile acids, nitrile esters, diesters, ester amides, amide acids, etc. which have been derived directly from these new compounds represent a large spectrum of physical properties and chemical reactivities and therefore constitute a large group of potentially useful

lubricant components. Stability studies which have just been completed indicate, however, that some of the compound types may find limited application because of undesirable lability toward strong acids and strong bases. Other new functional derivatives under active investigation are the β -hydroxy alkylamido or acylamido alkanes. These are prepared by the addition of alkyl or aryl carboxylic acids to epimino-alkanes. By-products of the reaction are 2-alkyl or 2-aryloxazolines. The two types of products, hydroxyamides and oxazolines, are interconvertible by hydration or dehydration. The hydroxy amides are of interest as lubricant components because of their hydrolytic stability, low volatility and crystalline properties. Oxazolines have been reported to have excellent stabilizing properties.

The thermal decomposition of ozonides is now visualized to proceed via a two-step mechanism. It was discovered that the free radicals formed during this reaction will serve as initiators for the polymerization of animal fats through their unsaturated chains.

The thermal decomposition of triolein ozonide in the presence of trilinolein represents a new method of preparation for dimer acid type products. Dimer acid derivatives have previously been shown to be lubricants of high thermal stability.

3. Detergents Investigations. Fat-based detergents, especially tallow-based alcohol sulfates, were shown to be superior to linear alkylbenzene-sulfonates under both aerobic and anaerobic conditions of biodegradation. Anaerobic conditions are important because about one-third of American dwellings use septic tanks, and because systems intended as aerobic sometimes cannot provide aeration for the waste load experienced.

Synthesis of a series of ether alcohol sulfates of the general formula $R(OCH_2CHR')_nOSO_3Na$ was completed. The sulfation product from hexadecanol and propylene oxide has good detergency and lime soap dispersing properties and is more easily soluble than the related tallow alcohol sulfates.

Alcoholysis of sodium methyl α -sulfostearate with sucrose, using sodium methylate catalyst, gave a product which appears to be a mixture of sodium sucrose α -sulfostearate with the starting components. A tallow-derived biodegradable α -sulfo fatty acid ester of sucrose may have use as a household detergent or as an industrial emulsifying agent.

In research at the University of Bombay, India, under a PL-480 grant, the reaction of an ester with acetone-glycerol, catalyzed by sodium methylate has been found to be an excellent method for the preparation of pure α -monoglycerides. Sulfation has been accomplished with pyridine- $-SO_3$. Surface active properties of sulfated monoglycerides and their combinations with soap are under investigation.

In contract research at Lehigh University, Bethlehem, Pa., physical-chemical studies on fat-based α -sulfo esters of the wetting agent (sodium

heptyl α -sulfopelargonate) and detergent (sodium methyl α -sulfopalmitate) types have been completed. Rates of adsorption onto carbon are more rapid for wetting agent types.

Reaction of benzene with oleic acid, methyl oleate, oleyl alcohol or oleonitrile gave phenylstearic acid and the corresponding ester, alcohol and nitrile. The products are viscous oils and are mixtures of 12 or 13 position isomers, a fact which makes them useful in lubricants, textile assistants, and as intermediates for easily soluble industrial surface active agents.

Contract research at Swift & Co., Chicago, has shown that oleic acid is a better starting material than methyl oleate or tallow for hydrogenolysis to oleyl alcohol, an intermediate for making detergents and surface active agents. A catalyzed, high-pressure hydrogenolysis of oleic acid gave 99.6% selectivity (% hydrogenolysis/% hydrogenation).

Under contract research at Archer-Daniels-Midland Co., Minneapolis, additive low temperature chlorination of tallow, methyl oleate and related compounds provided highly stable chlorine derivatives.

In PL-480-sponsored research at Centre National de la Recherche Scientifique, Paris, chlorination of saturated fatty acids and derivatives by (a) anodic electrolysis of aqueous sodium chloride, (b), electric discharge (sparking) in CCl_4 solution, and (c) photochlorination, are being studied. Products containing 20-55% Cl increase in viscosity from fluid yellow oils (20% Cl) to viscous masses (50% Cl - 8 Cl per molecule of stearic acid). Methyl stearate and stearic acid photochlorinated to high Cl content of about 70% was separated into a powder named "insoluble" and a glass-like product dubbed "glass." The latter has adhesive properties on hydrophilic and hydrophobic supports and has a high refractive index (1.8 at 20°C.). The "glass" is fairly stable but shows a small loss of chlorine on storage for 3 months, and decomposes with loss of HCl on heating to 100°C. The "glass" is chemically reactive, yielding a yellow oil on treatment with various chemical reducing agents, giving a highly unsaturated material on treatment with strong bases, and reacting rapidly with ethanolamine to give water soluble products.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

Chemical Composition, Physical Properties and Structure

- Barford, R. A., Bertsch, R. J., and Rothbart, H. L. 1968. Phase equilibria and countercurrent distribution. J. Am. Oil Chemists' Soc., 45, 141-143.
- Hunter, J. J., and Eddy, C. R. 1967. Dielectric properties of some long-chain esters in the solid state. J. Am. Oil Chemists' Soc., 44, 341-343.
- O'Connor, R. T., Allen, R. R., Chipault, J. R., Herb, S. F., and Hoerr, C. W. 1968. Report of the Instrumental Techniques Committee, AOCS, 1966-1967. J. Am. Oil Chemists' Soc., 45, 103-106.
- Purcell, J. M., and Susi, H. 1968. Proton magnetic resonance spectra of acetylenic fatty acids. Anal. Chem. 40, 571-575.
- Quinn, J. G., Sampugna, J., and Jensen, R. G. 1967. Synthesis of 100-gram quantities of highly purified mixed acid triglycerides. J. Am. Oil Chemists' Soc., 44, 439-442.
- Rabinowitz, J. L., Luddy, F. E., Barford, R. A., Herb, S. F., Orlean, S. L., and Cohen, D. W. 1967. Lipid determination in powdered human dentin by thin-layer and gas-liquid chromatography. J. Dental Res., 46, 1086-1089.

Chemical and Physical Investigations to Improve Products

- Ault, W. C., Eisner, A., Bilyk, A. and Dooley, C. J. 1967. Nonvolatile alpha-branched-chain fatty esters. J. Am. Oil Chemists' Soc. 44, 506-508.
- Ault, W. C., Parker, W. E., Eisner, A., Koos, R. E. and Knight, H. B. 1968. Bench-scale evaluation of nonvolatile alpha-branched chain fatty esters as potential lubricants. J. Am. Oil Chemists' Soc., 45, 70-71.
- Bistline, R. G., Jr., and Stirton, A. J. 1968. Benzyl, cyclohexyl and phenyl esters of alpha-sulfo fatty acids. J. Am. Oil Chemists' Soc., 45, 78-79.
- Boucher, E. A., Grinchuk, T. M. and Zettlemoyer, A. C. 1967. Measurement of surface tension of surfactant solutions. J. Colloid and Interface Sci., 23, 600-603.
- Boucher, E. A., Grinchuk, T. M., and Zettlemoyer, A. C. 1968. Surface activity of sodium salts of alpha-sulfo fatty esters: the air-water interface. J. Am. Oil Chemists' Soc., 45, 48-52.

- Cordon, T. C., Mauer, E. W., Nunez-Ponzoa, M. V., and Stirton, A. J. 1968. Metabolism of some anionic tallow-based detergents by sewage microorganisms. *Appl. Microbiol.* 16, 48-52.
- Lai, R., Naudet, M., and Ucciani, E. 1968. La synthese oxo em lipochimie. II. Etude complementaire de l'hydroformylation de l'oleate de methyle. *Rev. Franc. Corps Gras*, 15, 15-21.
- Maerker, G., Kenney, H. E. and Donahue, E. T. 1968. Cyanoethylation of hydroxy derivatives of fats. *J. Am. Oil Chemists' Soc.* 45, 72-75.
- Rothman, E. S. 1968. Reactions of the stearoyleated enolic form of acetone, involving hexadecylketene as the reactive intermediate, *J. Am. Oil Chemists' Soc.* 45, 189-193.
- Scholnick, F., Saggese, E. J., Wrigley, A. N., Ault, W. C., Monroe, H. A., Jr., and Zubillaga, M. 1968. Urethane foams from animal fats. IV. Rigid foams from epoxidized glycerides. *J. Am. Oil Chemists' Soc.*, 45, 76-77.
- Smith, F. D., Stirton, A. J. and Nunez-Ponzoa, M. V. 1967. Phenyloctadecanol and surface-active derivatives. *J. Am. Oil Chemists' Soc.* 44, 413-415.
- Smith, F. D. and Stirton, A. J. 1967. The alpha-sulfonation of alkyl palmitates and stearates. *J. Am. Oil Chemists' Soc.* 44, 405-406.
- Tubul, A., Ucciani, E., and Naudet, M. 1967. Sur l'obtention de derives hydroxyles allyliques des chaines grasses monoinsaturees. III. Oxydation par l'acetate mercurique. *Rev. Franc. Corps Gras*, 14, 13-19.
- Tubul, A., Ucciani, E., and Naudet, M. 1967. Sur l'oxydation de l'oleate de methyle par l'acetate mercurique. *Bull. Soc. Chim. France*, 464-467.
- Weil, J. K., Stirton, A. J. and Leardi, E. B. 1967. Ether alcohol sulfates from oleyl alcohol. *J. Am. Oil Chemists' Soc.* 44, 522-524.
- Zettlemoyer, A. C., Subba Rao, V. V., and Fix, Robert J. 1967. Rates of adsorption of wetting agents and detergents at 'graphon' solution interface. *Nature*, 216, 683.
- Rothman, E. S. March 26, 1968. Process for the preparation of stearone. U. S. Patent 3,375,281.
- Scholnick, F., Monroe, H. A., Jr., Wrigley, A. N., and Ault, W. C. June 6, 1967. Resins from hydroxyphenylstearic acid. U. S. Patent 3,324,059.

HIDES AND LEATHER UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y. 1968			
	: Research Problem Area			: Total
	: 411	: 410	: 702	:
Pennsylvania (Wyndmoor)	:	:	:	:
A. Chemical Composition, Physical Properties and Structure	: 2.1	: 5.9	: 0	: 8.0
B. Chemical and Physical Investigations to Improve Products	: 8.7	: 0	: 0	: 8.7
C. Microbiology and Toxicology	: 0	: 0	: 0.5	: 0.5
D. Technology - Process and Product Development	: 5.8	: 3.5	: 0	: 9.3
Total	:16.6	: 9.4	: 0.5	:26.5

Intramural program is supplemented by extramural support representing (a) 1.8 SMY's at universities and state agricultural experiment stations^{1,2/}, (b) 0.2 SMY's at other U.S. institutions^{2/}, and (c) PL-480 funds in 4 countries representing 459,000 U.S. dollars equivalent.

1/ RPA 411 - Chemical Composition, Physical Properties and Structure, 0.8;

2/ RPA 410 - Chemical Composition, Physical Properties and Structure, 1.2.

Problems and Objectives

Hides are an important product of the cattle industry, accounting for 6-10% of the live weight of the cattle. The U.S. is, however, now using less than two-thirds of the 33 million hides produced annually. The foreign markets that currently absorb the surplus hides are also threatened by an increased world hide production and a decrease in the use of leather. The traditional use of leather in shoe uppers is being replaced by synthetic polymeric materials.

Utilization research seeks to improve the market for hides by providing processes for more efficient production of leather with enhanced and new properties and by providing alternative uses for hides or hide pieces.

Major objectives of the research are:

1. Developing chemical and physical treatments for animal hides and leather to impart to leather products new and permanent strength properties and easy-care properties.
2. Increasing the value of hides, skins and leather by the elimination of defects.
3. Improving technology of processing hides to final products.
4. Providing new edible uses on a large scale for collagen from cattle hides.
5. Develop improved methodology that will permit more rapid and precise detection of Salmonellae in meat and bone meals.

Progress - USDA and Cooperative Program

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS

AND ANIMAL FATS

A. Chemical Composition, Physical Properties and Structure

1. Protein Constituents and Collagen. In the comparative work on collagen of different species, at the University of Turku, Finland, under a PL-480 grant, it was noted that the stability of collagen has improved during evolution and can be correlated with the changes in the primary structures of the peptide chains. Certain differences were also found between collagens from animals of various ages or from various tissues. The results can be utilized as a guide in the selection of the gelatinous materials for the eventual synthesis of artificial polypeptides.

Certain large fragments, which have been obtained with pepsin from soluble collagen, can be prepared pure and even localized in the tropocollagen macromolecule by the electron microscope. In addition, about ten small fragments (MW 5000-10000) can be prepared pure but they have not yet been characterized.

New fundamental knowledge was obtained on the effect of bivalent ions in the insolubilization of soluble skin collagen. The significance of the carbonyl groups is also confirmed. The age-dependent variation in the fractions liberated by stepwise heating from insoluble collagen was demonstrated.

In PL-480-sponsored research at the Central Leather Research Institute, Madras, India, grafting of acrylonitrile, acrylamide, and methyl methacrylate onto collagen was studied using ceric ion as an initiator. Analysis indicated considerable amount of polymerization. Attempts were made to demonstrate the existence and extent of grafting onto the protein. Evidence indicates that extent of grafting is of the order of 50 to 200 percent. Cyanoethylation of collagen was effected under various conditions. The maximum amount of cyanoethyl groups introduced was 0.4 moles per gram of collagen, corresponding to the amount of free amino groups in collagen.

These studies provide basic information needed for developing chemical and physical treatments for hides to provide improved leather products.

2. Hides and Leather. Specific ion electrodes have been employed to measure the concentration of acid and salt at the split surface of a hide. A flat surfaced glass electrode gave reproducible pH values of a hide surface under a variety of conditions. The pH of the supernatant solution is not always the same as that of the hide surface and squeezing of liquid from the hide can alter the equilibrium. A silver chloride electrode with a flat bottom accurately measures the chloride content of a hide surface and this is equivalent to the concentration of the supernatant solution. Squeezing of salt solution from the hide does not alter the equilibrium of the chloride ions. This electrode was used to measure the rate of penetration of salt solution into a hide. The movement of salt within a hide is dependent upon concentration, moisture, hide location, pH and degree of manipulation of the hide. Under static conditions water equilibrates in hides more rapidly than electrolytes.

By means of differential thermal analysis it was shown that the shrinkage or transition temperature of leather is decreased in a relatively short time as a result of cycling leather between various conditions of wetness and dryness. Higher drying temperatures accelerated the decrease in shrinkage temperature. Subsequent exposure of conditioned specimens to 100% relative humidity brought about a partial recovery from the loss in shrink temperature.

In research under a PL-480 grant at the Central Leather Research Institute, Madras, India, it was shown that different tannages impart different structural stability to hides and skins. Chrome-tanned skins shrank less

than vegetable tanned skins when heated in water above the shrinkage temperature. However, recovery of shrinkage was virtually nil. Fish oil and formaldehyde tanned skins recover their shrinkage losses to a considerable extent. Wattle and quinone (as known previously) give leathers with about the same Ts, however, recovery of shrinkage was considerably greater for quinone tanned as compared to wattle tanned collagen.

Under another PL-480 grant at the Central Leather Research Institute, measurements of the moisture absorbed by a calf upper leather sample placed at various locations within a shoe revealed that the highest moisture condition occurs near the ball of the foot and the lowest is just above the heel on the outside of the foot. Values above the toes and instep were intermediate. The trend was the same regardless of whether leather or rubber soles were used but the moisture absorbed was always greater for the rubber-soled shoes.

Work was also carried out on measuring the effect of materials leached from leathers by water and perspiration. A number of fungi were found to be present in a high percentage of the shoes studied. The fungi will grow better on sweat extracts of leather than on water extracts, however, some of the fungi found are known to produce antibacterial substances and these may be beneficial in shoes.

These studies provide basic information on hides and leather needed for developing chemical and physical treatments for hides to provide improved leather products and for improving processing technology.

B. Chemical and Physical Investigations to Improve Products

About 40 different cuprophenyl dyes were evaluated for leather from the standpoint of washfastness and perspiration resistance. Several of these dyes were quite good with regard to these properties, however, these dyes lacked good penetration. Acid reactive dyes and methylol reactive dyes were evaluated for leather. These reactive dyes gave leather products that were not fast to washing. Tests with chloro-s-triazine dyes showed that these dyes had good penetration, and that eight of these dyes were fast to washing. The use of glutaraldehyde in retanning the chrome leather appeared to aid the fixation of these dyes. In larger scale tests full skins were dyed and finished into suede garment leather. Preliminary laboratory tests indicated the colors to be fast to hydrocarbon solvents and perchloroethylene. A test in commercial equipment indicated the colors were fast to drycleaning with mixed fluorochemical solvent.

The hydration properties of various leathers under study showed that the application of water repellents did not drastically reduce the water vapor permeability of alkenyl succinic acid (ASA)-lubricated leathers. As the relative humidity increased so did the water vapor absorption of the various leathers under study. Leathers impregnated with water-repellent materials retain their affinity for water vapor and when compared to non-repellent leathers only slight differences in water vapor uptake were noted. Leathers

treated with water-repellent materials showed a reduced affinity for liquid water. The ASA-lubricated leathers which are highly absorbent to liquid water, gave up absorbed water much faster than leathers treated with water-repellent materials.

Application of a Mannich reaction using formaldehyde and malonic acid to hide protein increased the carboxyl groups in this protein by about one-third. Examination of hydrolysates of this modified protein revealed five new compounds each being a derivative of lysine. Two were established as the mono- and di-carboxyethylated derivatives of lysine. Skins modified in this manner and tanned with chrome showed a higher shrinkage temperature and less loss of chrome in washing and after exposure to synthetic perspirant solutions.

A method has been developed for determining formaldehyde and malonic acid, essentially quantitatively, in aqueous solution using gas-liquid chromatography. This method will be used in studies of the mechanism of the reaction of these two compounds with collagen. Glutaraldehyde in this Mannich system was less effective than formaldehyde.

This research on chemical modification is needed to impart to leather products permanent strength properties and easy-care properties such as colorfastness and water-repellency.

C. Microbiology and Toxicology (no current research)

D. Technology - Process and Product Development

1. Eliminating Defects of Hides and Leather. Under optimum conditions, freezebranding affects only the production of hair pigment, resulting in a white-haired brand. Freezebranding can also destroy the entire hair follicle and induce varying degrees of dermal scarring. From histological studies freezebranding was found to cause much less damage to leather than hot branding. The damage produced by firebrands may be minimized or eliminated through the development of freezebranding. Better visibility of smaller brands permits use of multiple-digit codes, making the method most attractive for detailed animal identification.

Studies on 59 woolskins confirmed that cockle defects are most serious during later winter and early spring and that the entire skin may be affected. There appears to be a relationship between the presence of sheep ticks, or keds, and the corresponding incidence of cockle. The serious losses in sheepskin leathers caused by cockle may be eliminated once the cause is determined and corrective measures established.

Impregnation of chrome-tanned calfskins with a 10% solution of large-particle-size cationic polyethylene emulsion reduces veininess. Zirconium sulfate in combination with the polyethylene also shows promise. Reduction of veininess in calfskin leather would upgrade the product in quality and price.

2. Other Pretanning and Tanning Investigations. In the research on dehydration of hides, more concentrated solutions of sodium chloride, as expected, improved the dehydration effect of butyl carbitol. Brine and 20% sodium sulfate, even without butyl carbitol, gave good fibrous dehydration products in tests with small pieces of hide. This principle of dehydration has been extended to stock in the tanned state resulting in leather with good fiber characteristics. In a cooperative test, dehydrated calfskin when processed to finished leather produced leather that was inferior to normal production as judged by the poor break. However, when compared to a control which was a brined calfskin, the experimental leathers appeared to show improved strength values. Improper rehydration of the dehydrated calfskin as well as improper fleshing of the fresh skins may be factors in obtaining variable results.

In research under a PL-480 grant at Leather Research Institute, T.N.O., Waalwijk, Holland, the effect of masked chrome liquors in chromium uptake by hide powder was investigated. Sodium salts of dicarboxylic acids (oxalate, adipate and phthalate) increased retention of chromium by hide powder. The phthalate had a pronounced effect on reaction velocity and chrome retention. Sodium salts of hydroxy acids (glycolate, lactate, or gluconate) lead to decreased chrome retention by hide powder in the order mentioned. Generally, the pH level of tanning increased with increasing amounts of the masking agents, due to their buffer action, except for glycolate, lactate or gluconate, in which pH decreased.

At the Central Leather Research Institute, Madras, India, under a PL-480 grant, progress has been made toward separating chromium tanning complexes on ion exchange columns. The separate fractions were analyzed, and the nature of the complexes involved partially revealed. Tanning studies with the separated fractions have not led to any clear-cut differences. This may be due to an equilibrium existing between the various species of complex. Some of these transformations have been studied in an isolated system, and the fastest change amounted to only 60% in 7 days. In the presence of ion exchange resin or hide collagen, there is some evidence that the transformations may be more rapid or may be driven in one direction by the removal of specific complexes.

Under another PL-480 grant to Central Leather Research Institute, Madras, two convenient laboratory tests to study rapid tanning of sole leather have been devised to confirm practical knowledge about the rate of penetration of vegetable tannins. Leaching at regular high temperatures did not impair the properties of wattle and babul. While a blend of natural tannins penetrated more slowly than individual tannins, a blend is desirable to give a balance in the properties conferred to vegetable-tanned leather. Sludge may be increased by the more rapid tanning and removal of the more soluble tannins. Buffered blends at a pH of about 4.5 - 5.0, formaldehyde pretanning and tanning in a sulfite liquor all speeded vegetable tanning for sole leather.

3. Products. Glutaraldehyde-chrome-tanned wool-on sheepskins were shown by a three-year use-test to be markedly superior to conventionally tanned shearlings to washing and alkaline solutions. Their durability now makes them economically feasible for medical pads and paint rollers. Their use is increasing.

Sheepskins tanned with glutaraldehyde alone and in combination with vegetable tannins have good resistance to the PIRA test (sulfuric acid and hydrogen peroxide). Investigations to determine the best fat liquor for this leather are being continued. Bookbinding leather which will resist the PIRA test would help increase domestic production. At present all PIRA-resistant leather is imported.

In comparison of chrome-tannage and glutaraldehyde-chrome-tannage of deerskin, differences between individual skins of the same tannage showed as much variation in puncture strength as between different tannages. Deerskin leather is being evaluated for use in handicraft work in impoverished areas.

The foregoing research is important to lower cost technology of leather manufacture by (a) eliminating defects in hides, and by (b) improvements in processing hides, including continuous processing.

RPA 410 - NEW AND IMPROVED MEAT, MILK AND EGG PRODUCTS

A. Chemical Composition, Physical Properties and Structure

In contract research, now completed, at the Midwest Research Institute, Kansas City, Missouri, cattlehides unhaired by a conventional lime-sodium sulfide method yielded collagen that was readily dispersed after mild acid treatment. This provides a practical source of raw material from whole hides or flesh splits. Dilute dispersions can be prepared at room temperature but 20-30% dispersions require heat. The collagen was precipitated by tanning agents, sodium chloride and chondroitin sulfate. Typical tropo-collagen structures were visible in all electron microscope pictures.

Apparatus was set up at EU to study the electric birefringence of solubilized collagen. This included the design and construction of a broad-band pulse amplifier to produce pulses of unusually large amplitude. A study of the electric birefringence of solubilized collagen using one-cycle square waves has shown that the rotatory diffusion constant has a concentration dependence and a time of storage dependence. Decreasing the ionic strength of collagen solubilized in citrate buffer to very low values results in a tendency toward syneresis. When a sample of calfskin corium was repeatedly extracted with citrate buffer the carbohydrate concentration decreased with each successive extraction although the collagen level remained constant.

Studies of the viscometric behavior of solubilized collagen were carried out under varying conditions of pH and ionic strength. The results indicate a definite electroviscous effect, and the data can provide information on the electric charge pattern of the collagen molecule. This, in turn, leads to

elucidation of the polymerization and aggregation processes of collagen.

Rotational isomerism of protein constituents in aqueous solution was investigated by nuclear magnetic resonance spectroscopy. The tyrosine anion rotamer energies are strongly dependent on temperature but not on concentration. Solute-solute as well as solute-solvent interactions play an important role in the conformational stability of aromatic amino acids. An NMR attachment that will permit the digitizing of spectral information and recording the data on paper tape has been assembled. The ability to increase the signal-to-noise ratio will extend the measurement capability to much more dilute samples and permit measurements on systems previously not amenable to investigation. The thermodynamics of amide-to-amide hydrogen bonding was investigated by NMR spectroscopy. Calculated bond energies are in some cases sensitive to extrapolated values of the chemical shifts of monomeric species. Best results are obtained if NMR and infrared measurements are carried out to provide complementary data. Infrared results suggest that "collagen films" contain a significant amount of material that is structurally different from native or properly reconstituted collagen, if molecular characteristics are used as a criterion.

In grant research at the Northwestern University School of Medicine, Chicago, acetyl glycine-N-methylamide was selected as a model polypeptide whose side chains are blocked so that all subsequent reactions are limited to the main polypeptide chain. After synthesis and characterization by NMR and IR, the interaction and transconformation reactions of this polypeptide in nonaqueous solvent pairs were studied via IR spectra. Gelatin films were deposited on silver chloride plates and then IR spectra and optical rotation were observed. The cis-trans isomerization of gelatin in the solvent pairs, trifluoroacetic acid - dimethyl sulfoxide, trifluoroacetic acid - dimethylformamide, and trifluoroacetic acid - acetonitrile was studied by optical rotation and viscosity measurements. Polymers of the repeating unit glycine - proline - proline were prepared with the end in view of using them as model compounds for polymeric gelatins. Potential energy maps showing the permitted configurations of that sequence were plotted. These polypeptide studies help elucidate the biosynthesis of collagen in vivo and its reconstitution from the solubilized state. These studies are providing basic information needed for developing new food uses for collagen.

- B. Chemical and Physical Investigations to Improve Products (no current research)
- C. Microbiology and Toxicology (no current research)
- D. Technology - Process and Product Development

In the development of food uses for collagen, some promising results have been obtained in experiments to produce a film, which when used as a coating for ground meat, will stand cooking temperatures. Use of commercially-unhaired fresh hide appears to have eliminated an "off" flavor found in cured stock. A process is being developed to granulate frozen hide at about

40% solids.

Films have been made also from equal amounts of soya meal and acid-dispersed collagen.

A procedure was developed for converting "limed" fresh splits of cattlehides into granular dry collagen. Rat-feeding tests proved this product digestible and nontoxic.

Collagen is being tested as an extender for ground meat, with encouraging results.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

- A. Chemical Composition, Physical Properties and Structure (no current research)
- B. Chemical and Physical Investigation to Improve Products (no current research)
- C. Microbiology and Toxicology

A method for detecting urea breakdown by Proteus sp. has been devised, using disposable plastic multicompartmented trays and stab inocula from colonies growing on brilliant green agar plates. This method reduces labor and equipment requirements and shortens the test time for differentiation of Salmonella from Proteus inasmuch as the test can be performed directly from isolated colonies without prior transfer.

- D. Technology - Process and Product Development (no current research)

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

RPA 411 - NEW AND IMPROVED PRODUCTS FROM WOOL, HIDES, SKINS AND ANIMAL FATS

Chemical Composition, Physical Properties, and Structure

Kedlaya, K. J., Ramanathan, N., and Nayudamma, Y. 1967. Studies on the shrinkage phenomenon: X. Effect of using processing liquor as heating medium on the shrinkage behaviour of skins subjected to pretanning treatments. *Leather Sci.* 14, 321-325.

Kedlaya, K. J., Ramanathan, N., and Nayudamma, Y. 1968. Studies on the shrinkage phenomenon: XI. Effect of pretanning treatments on shrinkage properties of collagen fibres. *Leather Sci.* 15, 40-45.

Kedlaya, K. J., Ramanathan, N., and Nayudamma, Y. 1968. Studies on elastoidin. III. Role of disulphide linkage in the hydrothermal shrinkage of elastoidin. *Leather Sci.* 15, 6-9.

Chemical and Physical Investigations to Improve Products

Feairheller, S. H., Taylor, M. M., and Filachione, E. M. 1967. The Mannich reaction with malonic acid and formaldehyde as a pretreatment for mineral tannages. *J. Am. Leather Chemists' Assoc.*, 62, 408-422.

Filachione, E. M., Korn, A. H. and Ard, J. S. 1967. The ultraviolet absorption of protein-bound glutaraldehyde. *J. Am. Leather Chemists' Assoc.*, 62, 450-453.

Ghosh, D., Vijayalakshmi, K., and Nayudamma, Y. 1967. A note on the properties of the extract of goran wood. *Leather Sci.* 14, 128-129.

Ghosh, D., Vijayalakshmi, K., and Nayudamma, Y. 1967. Studies on the effect of nontans in vegetable tanning. *Leather Sci.* 14, 154-160.

Korn, A. H., and Filachione, E. M. 1967. The direct determination of bound glutaraldehyde in glutaraldehyde-tanned collagen. *J. Am. Leather Chemists' Assoc.* 62, 507-522.

Santhanam, P. S., Ghosh D., and Nayudamma, Y. 1967. Oxidative and degradative changes occurring in babul liquor. *Leather Sci.* 14, 179-185.

Vijayalakshmi, K., Santhanam, P. S., and Nayudamma, Y. 1967. A note on the keeping quality of babul liquor. *Leather Sci.* 14, 331-332.

Fein, Martin L., and Filachione, Edward M. October 10, 1967. Tanning with a condensation product of acrolein and formaldehyde. U. S. Patent 3,346,324.

Technology - Process and Product Development

- Ghosh, D., Thampuran, K. R. V., and Balakrishnan, M. 1967. Studies on the leaching systems of some vegetable tanning materials. *Tanner*, 21,309-315.
- Happich, William F. 1968. Home tanning of woolskins with glutaraldehyde. *The Shepherd*, 13, (3), 16-20.
- Happich, W. F., Windus, W., and Naghski, J. 1967. The moisture sorption properties of glutaraldehyde-chrome-tanned shearlings. *J. Am. Leather Chemists' Assoc.* 62, 718-732.
- Happich, M. L., Naghski, J., and Windus, W. 1968. The application of glutaraldehyde to tanning belly work glove leather. *J. Am. Leather Chemists' Assoc.* 63, 74-89.
- Khanna, J. K., Nandy, S. C., and Nayudamma, Y. 1967. Leather making potentiality of cattle hides obtained from fallen (dead) and slaughtered animals. III. *Leather Sci.* 14, 295-299.
- Khanna, J. K., Nandy, S. C., and Nayudamma, Y. 1967. Effect of sun drying cattle hides on the quality of chrome upper leather. *Leather Sci.* 14, 326-330.
- Tancous, Jean J., Schmitt, Rolf, and Windus, W. 1967. Pulpiness and weakness in side upper leather as related to fresh hides. *J. Am. Leather Chemists' Assoc.* 62, 781-807.
- Thampuran, K. R. V., Balakrishnan, M., and Ghosh, D. 1967. Effect of pre-conditioning the pelt to different pH levels on heavy leather tannage. *Leather Sci.* 14, 263-267.
- Windus, W. 1967. Retanning chrome-tanned leather. *Leather Mfr.* 84, no. 10, 47-51.
- Happich, W. F., Windus, W., and Naghski, J. September 11, 1967. Glutaraldehyde stabilized wool. U. S. Patent 3,342,543.

POTATO UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y. 1968		
	: Research Problem Area		:Total
	: 403	: 901	:
Pennsylvania (Wyndmoor)	:	:	:
A. Chemical Composition and Physical Properties	: 4.1	: 0	: 4.1
B. Color, Texture and Other Quality Factors	: 4.1	: 0	: 4.1
C. Technology - Process and Product Development	: 2.0	: 2.5	: 4.5
Total	: 10.2	: 2.5	:12.7

Intramural program is not currently supplemented by extramural support.

Problem and Objectives

The welfare of the potato industry depends on continuing the present trend to use increasing amounts of the potato crop for processing into products such as chips, French fries and dehydrated potatoes. Processing of potatoes, however, poses several problems, the most important being disposal of potato wastes. Antipollution laws may force some plants out of business, particularly the potato starch plants which provide growers and processors with an outlet for cull potatoes. Another area of concern relates to predicting and controlling processing characteristics of potatoes. In order to extend the processing season through the winter months, fall-harvested potatoes are generally placed in cold storage where excessive amounts of sugar accumulate, requiring a lengthy reconditioning treatment before processing. No potato variety can be chipped directly out of storage. Some cannot be reconditioned successfully. Research is also needed to provide new and improved processed potato products.

Major objectives of present research are:

1. Developing technology for recovery of protein and amino acids from potato waste.
2. Developing technology for evaluating potatoes for processing, controlling browning during processing, and predicting quality at time of consumer use.
3. Perfecting the explosion-puffing process to provide new and improved dehydrated potato products.

Progress - USDA and Cooperative Programs

RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

A. Chemical Composition and Physical Properties

1. Potato Lipids. An "unknown" fraction of fatty acids found in relatively great concentration in immature tubers has been shown to be a mixture of three unsaturated C18 acids and the C-23 and C-24 saturated acids. The fraction containing these acids decreases to zero in mature tubers.
2. After-cooking Discoloration. In any lot of potatoes, a direct correlation was found between degree of after-cooking blackening and size of tuber. The following characteristics, which are more likely in larger tubers, favor discoloration: (a) low organic acid content, especially citric; (b) high potassium/citric acid ratio; (c) high polyphenolic content; (d) high sodium content; (e) low citric acid/polyphenolic ratio. Specific gravity is not related to blackening.
3. Potato Proteins. Conditions have been established permitting replication of disc electrophoresis patterns for potato tuber proteins. A study of

variability within tubers of the same plant, row, field, and area and variety has indicated that each tuber variety does have a characteristic pattern, though differences are sometimes minor. A survey of the range of protein and free amino content of potato varieties is in progress.

4. Reducing Sugars and Enzyme Activity in Stored Potatoes. Potato tuber invertase inhibitor has been characterized; it inhibits several other plant invertases and may be useful for invertase classification. Biochemical analysis has identified potato clones with unusually low reducing sugar after storage. Progeny will be analyzed to determine transmission of the desired characteristic. Selected seedlings are being assayed for sucrose synthetase, amylase, invertase and its inhibitor. Agricultural carbamates and mild virus x infection do not affect sugar transformations.

This fundamental research on composition and properties of potatoes is needed as a basis for evaluating potatoes for processing and for controlling color and other properties during processing.

B. Color, Texture and Other Quality Factors

1. Frozen French-fried potatoes. Both crust shear and area under the entire shear curve of French-fried potatoes are proportional to specific gravity of the raw stock, though such differences cannot be detected by a panel. A great deal of data on potato samples of varying origin and history must be accumulated before a shear test can be useful for predicting quality of frozen French-fried potatoes after storage.

2. Pigments Formed in Potato Frying. The use of microwave heating to finish potato chips reduces loss of amino acids and sugars by one-half and one-third, respectively, as compared to conventional fat finishing. While intermediate browning compounds were present in considerable quantities in the microwave finished chips, the production of brown pigments was diminished.

Work continues on use of a model system to examine intermediates in amino acid and sugar reactions under chip frying conditions. Techniques for isolating intermediates have been developed using a product of the isoleucine-glucose reaction. Gamma-amino butyric acid is the most reactive amino acid with glucose.

C. Technology - Process and Product Development

1. Quick-cooking Dehydrated Potato Products. It has been determined that the off-flavors which sometimes develop in the explosive-puffing process for potatoes are probably due to aldehydes formed from the Strecker degradation, a reaction between certain amino acids and sugars, and that these off-flavors develop in the gun. Attempts to block the reaction by reacting the carbonyl group with calcium or magnesium ions were unsuccessful. Soaking potato dice 1 hour in water at 160°F. removed enough of the reactants to reduce the off-flavors below the taste threshold but the product lacked potato flavor and nutrients. Addition of amino acids and like substances, in the hope that

they might react with the sugars more selectively than the natural amino acids of the potato and form end products less disagreeable than the aldehydes heretofore formed, showed no improvement. Other means of blocking the Strecker degradation are under study.

2. Oil Content of Potato Chips. A significant reduction in oil content of potato chips has been obtained by modification of the pilot plant chip fryer. The potato chips are fried at a higher temperature and the lower viscosity of the hotter oil favors more complete drainage of oil from the finished chip. The changes in design of the potato chip fryer may be of commercial interest.

RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

- A. Chemical Composition and Physical Properties (no current research)
- B. Color, Texture and Other Quality Factors (no current research)
- C. Technology - Process and Product Development

1. Protein and Amino Acids from Potato Waste. Laboratory-scale equipment for ion exchange removal of amino acids from simulated potato starch processing waste has been installed and preliminary runs made. Reverse osmosis equipment for concentrating such waste has been acquired and preliminary tests indicate that the desired degree of concentration can be attained.

Publications and Patents - USDA and Cooperative Programs

RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Chemical Composition and Physical Properties

- Pressey, R. 1967. Purification and properties of phosphoglucomutase from potato tubers. J. Food Sci., 32, 381-385.
- Pressey, R. 1967. Invertase inhibitor from potatoes: Purification, characterization, and reactivity with plant invertases. Plant Physiol. 42, 1780-1786.
- Schwartz, J. H. 1967. Apparatus and procedure for developing thin-layer plates under nitrogen. J. Chromatog., 30, 619-621.
- Schwartz, J. H., Fow, M. I., Raskin, R. L., and Porter, W. L. 1968. Chemical composition of potatoes. VI. Effect of variety and location on acid concentrations. Am. Potato J., 45, 81-92.
- Zacharius, R. M., and Porter, W. L. 1967. Further studies on the behavior of some non-nitrogenous compounds with ninhydrin during automatic ion exchange chromatography. J. Chromatog., 30, 190-197.
- Zaehring, M. V., Reeve, R. M., Talley, E. A., Dinkle, D. H., and Hyde, R. B. 1967. Specific gravity and composition of potatoes for various processing and cooking purposes. Potato Assoc. of Am. Potato Handbook, 12, 5-10.

Color, Texture and Other Quality Factors

- Butchbaker, A. F., Nelson, D. C., and Shaw, R. 1967. Controlled-atmosphere storage of potatoes. Trans. of Am. Soc. Agr. Engineers, 10, 534-538.
- Fitzpatrick, T. J. and Porter, W. L. 1968. Microwave finishing of potato chips: effect on the amino acids and sugars. Am. Potato J., 45, 103-110.
- Talley, E. A., and Porter, W. L. 1968. New quantitative approach to the study of non-enzymatic browning. J. Agr. Food Chem. 16, 262-264.

Technology - Process and Product Development

- Shaw, R. 1967. Red River Valley standard chip cooker is ready for 1967 crop. Valley Potato Grower, 19, no. 19, 12.

RPA 901 - ALLEVIATE SOIL, WATER, AND AIR POLLUTION

Technology - Process and Product Development

Shaw, R., 1966. Recovery of edible and industrial products from effluent streams of potato processing plants. 21st Purdue Ind. Waste Conf. Proc. 13-18.

VEGETABLE UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y.1968 :	
	: Research Problem Area	: Total
	403	
Pennsylvania (Wyndmoor)		
A. Technology - Process and		
Product Development	2.5	2.5
Total	2.5	2.5

Intramural program is not currently supplemented by extramural support.

Problems and Objectives

Economic and social factors are accelerating the trend towards processing greater amounts of the vegetable production. Utilization, as processed rather than fresh vegetables, provides a constant source of supply with less price fluctuation. Mechanical harvesting of vegetables minimizes the need for temporary farm labor forces.

Emphasis in research is on improvement in processing technology, particularly for dehydrated products. Consumer preference is shifting to "convenience" foods. Modern military feeding needs products with high bulk density which do not require refrigeration and are rapidly rehydratable.

Many dehydrated vegetable products rehydrate extremely slowly, and are limited in size, restricting utility of the products.

The major objective of this research is to perfect the explosion-puffing process to provide dehydrated vegetables which are rapidly rehydratable, cook in the same or less time than their fresh counterpart, and have good initial flavor and storage stability at room temperature.

Progress - USDA and Cooperative Programs

RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

A. Technology - Process and Product Development

1. Quick-Cooking Dehydrated Vegetable Pieces

Research on the batch explosive-puffing of celery has been continued. The Pascal variety, both California and Florida grown, has been used as raw material. Water-blanching in 1/2% sodium bicarbonate solution (pH 8.2 to 8.6) is essential in retaining the green color. Celery from both areas yielded a product of excellent color, rehydrating in 4 minutes simmering in water. The Florida variety, however, was not acceptable because of toughness. The California variety had excellent texture and was tender. Favorable operating conditions for the batch process have been determined.

2. Dehydrated Mushroom Products

Dehydration studies on mushroom pieces have resulted in the development of an economical method of making a dry product of acceptable flavor and color. Drying temperatures as high as 200°F. had very little or no effect on initial flavor and storage stability. Of all the chemicals commonly used for controlling color, only sodium bisulfite was found to give satisfactory results and then under carefully controlled conditions of concentration.

Publications and Patents - USDA and Cooperative Programs

RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Technology - Process and Product Development

Komanowsky, M., Turkot, V. A., Talley, F. B., and Eskew, R. K. 1966.

Drum-dried mushroom powder--A new product. U. S. Agricultural Research Service, ARS-73-53.

DECIDUOUS FRUIT AND TREE NUT UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y. 1968:	
	: Research Problem Area	:Total
	: 403	:
Pennsylvania (Wyndmoor)	:	:
A. Chemical Composition and Physical Properties	:	:
	: 0	: 0
B. Flavor	: 1.0	: 1.0
C. Color, Texture and Other Quality Factors	:	:
	: 1.8	: 1.8
D. Technology - Process and Product Development	:	:
	: 4.9	: 4.9
Total	: 7.7	: 7.7

Intramural program is supplemented by extramural support representing 0.8 SMY's at universities and state agricultural experiment stations^{1/}.

^{1/} RPA 403 - Chemical Composition and Physical Properties, 0.1, and Color, Texture and Other Quality Factors, 0.7.

Problems and Objectives

Continued improvement in the quality of processed fruits is essential if fruit is to hold its own as an element in the national diet. Mechanical harvesting and other changes in the growing and harvesting of fruits create problems which must be met by improved processing methods. The preservation of fruit juices with good flavor, color, and other properties is essential in maintaining good markets for these products. The development of new fruit varieties more suitable for various types of processing is an important factor in preserving markets for the fruit processing industry and protecting fruit growers against variations in price due to irregular yields from year to year.

Objectives of the research are:

1. To extend the shelf life of fresh cider without the use of chemical preservatives.
2. To develop high quality dehydrated fruit products which may be reconstituted quickly, and which may be consumed as snacks.
3. To develop an instant apple sauce of good quality from explosive-puffed dehydrated apples.
4. To develop improved methods for the processing of mechanically harvested fruits and for maintaining the processing quality of fruits harvested by this method.
5. To evaluate the processing characteristics of new fruit varieties.

Progress - USDA and Cooperative Programs

RPA 403 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

A. Chemical Composition, Physical Properties and Structure

In grant research at Temple University on the metabolism of bruised and unbruised cherries, studies were repeated using fresh fruit from 1967 crop. Mature cherries were "fed" C^{14} -labeled compounds (glucose, acetate and citrate) through the attached stem. After suitable holding periods, the fruits were inactivated by macerating in hot ethanol. The alcohol extracts and the dried insoluble solids were stored for further study. The various extracts and residues are being fractionated in order to determine where the C^{14} activity resides.

B. Flavor

Thirty components of cherry essence have been identified. Numerous other components have been detected, many of which have pronounced odor

characteristics. The carbonyl components of cherry essence are now being examined.

C. Color, Texture, and Other Quality Factors

In contract research at Rutgers University, a total of 27 new pear varieties of apparent blight resistant and desirable horticultural quality were harvested, stored, ripened and processed by canning. The raw fruit was evaluated, and after 4 to 6 months of storage the processed fruit will be examined.

Several new cherry harvesters have been evaluated for effect on product quality. In general, bruise damage was high and quality low. An estimated \$1,000,000 was lost due to reduction in quality. A procedure for reducing bruising by transferring some processing operations from the plant to the orchard has been developed. In 1967 the joint AERD-EURDD effort in mechanically harvesting of cherries is estimated to have saved \$3,000,000 in labor costs and permitted about \$9,000,000 worth of cherries to be harvested in areas where the crop was too light to be hand-harvested.

D. Technology - Process and Product Development

Explosion puffing has been successfully applied to the dehydration of York Imperial and Golden Delicious apple pieces. Dried applesauce made from a blend of these varieties together with sugar and citric acid and pie segments of York Imperial are being prepared for institutional market tests.

The substitution of sucrose for the monosaccharides in apples has made possible the preparation of explosion-puffed apple snacks which retain their crispness well.

A commercial 6-lamp unit for ultraviolet irradiation of cider, made to our specifications, was purchased and evaluated under commercial conditions. This process extends shelf life without heat or added preservatives. The availability of such equipment should hasten the adoption of the process by cider makers, and encourage the sale and distribution of fresh apple cider by dairies, since it need contain no added preservative.

The reduction of time for lye-peeling of apples obtained by the use of wetting agents is independent of that obtained by our alcohol dewaxing step, so that lye exposure can be reduced to 2 minutes or less at 140°F., using both. Calyx removal from the product is still a problem.

Publications and Patents - USDA and Cooperative Programs

RPA 4-3 - NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS

Color, Texture and Other Quality Factors

- DiMarco, G. R., and Gustafson, L. L. 1967. Evaluation of peaches for processing. U. S. Agricultural Research Service, ARS-72-57.
- Gaston, H. P., Levin, J. H., and Whittenberger, R. T. 1968. Ten years of progress in machine-harvesting fruit -- where we are, and where we go from here. Mich. State Hort. Soc., Annual Report, 97, 54-59.
- Gaston, H. P., Whittenberger, R. T., and Levin, J. H. 1968. Research group reports on machine-harvesting of Michigan grown sweet cherries. The Great Lakes Fruit Growers News, 7, No. 3, 14-16.
- Tennes, B. R., Levin, J. H., Diener, R. G., and Whittenberger, R. T. 1967. Firmness and pitter loss studies on tart cherries. Am. Soc. Agr. Eng., Paper 333, 14 pp.
- Whittenberger, R. T. 1968. How to control cherry scald in 1968. New York State Hort. Soc., Proceedings, 113, 117-120.
- Whittenberger, R. T., Harris, Margaret B., Hills, C. H., and Levin, J. H. 1967. Many factors affect cherry scald. Canner/Packer, 136, No. 7, 34.
- Whittenberger, R. T., Levin, J. H., and Gaston, H. P. 1968. Following cherry quality from the tree to the can. Mich. State Hort. Soc., Annual Report, 97, 65-69.

Technology - Process and Product Development

- Eisenhardt, N. H., Cording, J., Jr., Eskew, R. K., and Heiland, W. K. 1968. Dehydrated Explosion-puffed apples. U. S. Agricultural Research Service, ARS-73-57.

TOBACCO UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	:Scientist Man-Years F.Y. 1968		
	: Research Problem Area :	Total	
	: 709 :	407	:
Pennsylvania (Wyndmoor)	:	:	:
A. Chemical Composition, Physical Properties and Structure	:	:	:
	: 8.9 :	0.2	: 9.1
B. Chemical and Physical Investigations to Improve Products .	:	:	:
	: 6.1 :	0	: 6.1
C. Microbiology and Fermentation	:	:	:
	: 4.2 :	0	: 4.2
D. Technology - Process and Product Development	:	:	:
	: 1.0 :	0	: 1.0
Total	: 20.2 :	0.2	: 20.4

Intramural program is supplemented by extramural support representing (a) 17.1 SMY's at universities and at state agricultural experiment stations^{1/}, and (b) 3.0 SMY's at other U. S. institutions^{1/}.

^{1/} RPA 709 - Chemical Composition, Physical Properties and Structure, 4.2; Chemical and Physical Investigations to Improve Products, 10.0; and Technology - Process and Product Development, 5.9.

Problems and Objectives

Tobacco is grown on about a million acres, and in seven states typically provides more farm cash receipts than any other field crop. The most serious problem affecting the tobacco industry is the concern regarding effect on health.

Major shortcomings in developing a safer cigarette are the inadequacies of biological testing procedures and the failure to know which substances in tobacco and smoke should be removed. There is no practical procedure for determining which substances in tobacco and tobacco smoke are injurious to public health. Based on limited knowledge, some currently marketed cigarettes with charcoal and other filters apparently show selective reductions in certain properties in laboratory animals but cannot be considered a final solution. In addition, such cigarettes are deficient in flavor and aroma and meet with some consumer resistance. This problem requires a concerted effort to learn specifically what must be removed from cigarette smoke and how this can be done effectively without loss of desirable organoleptic properties.

Objectives of research are to:

1. Develop methods for determining which substances in tobacco and tobacco smoke are undesirable from standpoint of public safety, and
2. Develop technology for elimination of substances determined to be undesirable.

Progress - USDA and Cooperative Program

RPA 709 - REDUCTION IN HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

A. Chemical Composition, Physical Properties and Structure

1. Composition of Tobacco Smoke. A quinone, 2,3,6-trimethyl-1,4-naphthoquinone was isolated for the first time from cigarette smoke. This compound was obtained in very small quantity from the nitromethane-soluble neutral fraction of smoke condensate and was identified by spectral characteristics and by similarity to the synthesized compound. 9-Fluorenone, recently reported in smoke from dark tobaccos by French workers, was identified also as a component of this neutral fraction. A fractionation procedure was developed which enables considerably greater concentration of the polynuclear hydrocarbons than was possible heretofore. Biological tests can now be initiated on the subfractions of the neutral fraction to determine the role of polynuclear hydrocarbons, and other neutral compounds, in carcinogenic and cocarcinogenic activity.

The isolation of four aromatic amines from cigarette smoke condensate, previously reported, prompted a search for the highly carcinogenic beta-naphthylamine. This compound was not found. On the basis of model recovery experiments it was concluded that beta-naphthylamine, if present at all, does not occur in amounts greater than 0.04 μg per cigarette.

A basic pigment isolated from cigarette smoke condensate was shown to differ from the previously isolated acidic pigment in content of quinic acid and hydrolysis products (amino acids and nitrogenous bases).

At the University of Kentucky Research Foundation, Lexington, Kentucky, under contract research, cigarette smoke condensate was divided into a volatile fraction and a nonvolatile fraction by distillation at reduced pressure. These fractions were separated into a water-soluble and a water-insoluble fraction. The basic fraction was separated from the water-insoluble fraction and subjected to gas chromatographic analysis. About 50 compounds eluted prior to nicotine and at least 62 compounds after nicotine. Pyrrolidine, pyridine, pyrrole, picolines, lutidines, 3-vinylpyridine, 3-ethylpyridine, 2,4,6-collidine, 3-acetylpyridine, and quinoline were tentatively identified.

In research under another contract with University of Kentucky Research Foundation, a method was developed for determining the amounts of anthracene and pyrene in cigarette smoke. Smoke condensate was separated on a silic acid column. The first fraction (band) was further chromatographed on Sephadex LH-20, and the amounts of anthracene and pyrene were determined by gas chromatography. In the smoke of one hundred, 85 mm., unfiltered cigarettes were found 20.3 μg of anthracene and 21.5 μg of pyrene. Eight other previously reported polynuclear hydrocarbons were also identified by this method.

Also, at Lexington, Kentucky, potassium antimonate and potassium carbonate plus potassium hydroxide columns were employed for the first time in gas-solid chromatography of polynuclear aromatic hydrocarbons. The column containing $\text{K}_2\text{Sb}_4\text{O}_7$ gave good separation of an authentic mixture of phenanthrene, anthracene, 1-methylphenanthrene, 2-methylphenanthrene, and pyrene. Good separations of anthracene and phenanthrene were achieved on both the potassium antimonate and potassium carbonate plus potassium hydroxide columns. The amounts of phenanthrene and anthracene present in a cigarette smoke condensate fraction were determined gas-chromatographically using the antimonate column. In the smoke of one hundred 85 mm. commercial cigarettes without filter tip were found 39.6 μg of phenanthrene and 13.0 μg of anthracene.

Seven different nitrosamines were synthesized and their spectroscopic and gas chromatographic patterns were determined. The stability and the distribution coefficient of dimethylnitrosamine under conditions normally encountered in the fractionation of cigarette smoke condensate were investigated. Distribution coefficients of three other nitrosamines between ether and water were also determined. Supplementary data indicated that the occurrence of nitrosamines in the low boiling fraction of cigarette smoke condensate, or a steam volatile fraction of smoke condensate, was either nil or limited to

trace quantities.

2. Composition of Tobacco Leaf. The brown tobacco pigments were isolated from a large-scale extract of Turkish tobacco. Potassium hydroxide fusion of the nondialyzable fraction of the pigment yielded a variety of volatile bases and a silicone. Compositional studies on the leaf pigments of the four principal cigarette tobacco types have shown the pigments to be similar in physical and chemical characteristics. Studies were also initiated on the phenolic compounds of tobacco leaf. Extracts of flue-cured tobacco have been prepared and submitted for bioassay tests at the Roswell Park Memorial Institute, Buffalo, New York. Solvents of increasing polarity (petroleum ether, chloroform, acetone, ethanol, methanol, water) were used in the consecutive extractions.

In contract research at the Research Triangle Institute, Durham, North Carolina, the glyceryl ester fraction of the hexane extract was separated from the steryl ester fraction by gel permeation. At least 1% of the hexane extract consists of the glyceride fraction whereas the steryl esters make up a minimum of 1.5% of this extract. Acids in the glyceride fraction were tentatively identified as myristic, palmitic, stearic, oleic, linoleic, and linolenic by gas chromatographic analysis of the methyl esters. These studies indicate that the neutral resins of leaf contain many components capable of serving as potent precursors of polynuclear aromatic hydrocarbons (PAH) of smoke. On pyrolysis, the isolated sterols and cyclic triterpenes may easily fragment, dehydrogenate and react with other radicals or groups to give PAH and thus contribute disproportionately to the overall PAH levels of smoke. The presence of free α - and β -amyrin and free or esterified cholesterol in leaf has not been reported previously.

B. Chemical and Physical Investigations to Improve Products

1. Cigarette Modifiers. Cooperative research at the University of Kentucky, Lexington, has shown that the addition of various chemical additives to tobacco results in alterations of the levels of certain smoke constituents. Additives include chemicals such as flame-retardants, free-radical scavengers, oxidants and temperature depressants. Some changes in the level of possible health-related smoke constituents have been detected. Decreases of 27% for total particulate matter (tar); 42% for nicotine; 34% for phenol and 71% for benzo(a)pyrene were obtained. For the majority of modifiers evaluated thus far, the most consistent alteration is the reduction in total particulate matter.

Cigarettes containing 8.3% NaNO_3 were prepared and smoked on a newly-constructed smoking apparatus. The smoke (vapor phase) generated from these cigarettes was collected in an appropriate collection flask and analyzed by gas chromatographic and/or colorimetric methods. The addition of NaNO_3 to cigarettes was shown to produce relatively large quantities of undesirable vapor phase constituents such as nitrogen oxides, acetaldehyde, acrolein and acetonitrile. All of these increased by at least a factor of two in smoke from treated cigarettes (vs. control). The smoke of treated cigarettes in

addition contained 160 ppm of N_2O , whereas the quantity present in smoke from control cigarettes was too low to be detected. Addition of $NaNO_3$ also had the effect of increasing levels of olefinic hydrocarbons in smoke, while levels of CO, HCN, HCHO and paraffins remained unchanged. Other effects of $NaNO_3$ addition were (1) reduction of H_2S levels in smoke from 4.5 to 0.3 $\mu g/puff$ and (2) lowering of burn temperature from 850°C. to 788°C.

2. Pyrolytic Products. Pyrolytic studies at EU indicated some of the non-volatile acids in tobacco leaf may serve as phenol precursors. Sodium lactate gave relatively high yields of phenols. Thus far, many types of organic materials gave rise to phenols (phenol, o-cresol, m- and/or p-cresol, and others) under appropriate pyrolytic conditions. Tobacco leaf pigment yielded a variety of N-heterocycles but no nicotine. It appears that in a burning cigarette, other processes (e.g. distillation) in addition to pyrolysis influence smoke composition. Polynuclear aromatic hydrocarbons (PAH) can be formed from any compound containing C and H above temperatures of 600°C. Pyrolyses in air formed more complex PAH mixtures than those conducted in nitrogen. Cinnamic acid and similar compounds produced t-stilbene as a major component and at least 30 other compounds which were mainly PAH. A method was developed for analyzing many acids in pyrolysates as well as in tobacco products.

In contract research at the University of Kentucky Research Foundation, pyrolysis of lysine monohydrochloride and phenylalanine at 850°C. in nitrogen yielded small amounts of phenol. Lysine monochloride, leucine, phenylalanine, and tryptophan formed, upon pyrolysis, naphthalene, acenaphthalene, fluorene, fluoranthene, pyrene, benzofluorenes, chrysene, benzo(a)pyrene, anthracene, phenanthrene, picene, and at least six other polynuclear hydrocarbons. Low molecular weight compounds such as methane, ethylene, acetylene, and carbon monoxide were also formed. Composition of the pyrolytic products was dependent upon the temperature of pyrolysis and the starting material. Yields of polynuclear hydrocarbons varied directly with pyrolysis temperature whereas heterocyclic bases were formed in highest yield at the lower temperatures.

Glutamic acid, proline, serine, alanine, 2-aminobutyric acid, valine, and phenylethylamine were pyrolyzed at 650° and 850°C. Yields of condensate varied inversely with the temperature of pyrolysis, but there was no consistent trend between pyrolysate yield and the weight of constituent fractions (phenols, acids, bases). Yields of these constituent fractions and of the individual gaseous products were apparently more dependent upon amino acid structure. HCN was formed in highest yield from amino acids having branched chains or numerous functional groups.

3. Biological Assay Methods. In the development of new and improved biological assay methods under contract research at the University of Kentucky Research Foundation, studies on tracheal rings grown in tissue culture have demonstrated cellular responses to acetone-soluble smoke condensate and a partial recovery upon cessation of toxicant administration. The water-soluble smoke extract has shown ciliostatic properties which are concentration-related. It may be possible to develop a whole family of ciliostatic

dose-response curves for different fractions of the water-soluble smoke extract for preliminary screening of tobacco products.

Epithelial cell turnover rate is being investigated with two strains of mice. One strain exhibits a low incidence and the other a high incidence of spontaneous lung tumors. In order to avoid the possibility that the data may be biased due to the method employed to count the dividing cells, two different methods are being used. One of these employs colchicine to arrest the cells in metaphase; the other utilizes tritiated thymidine to label those cells in the process of dividing. In order to obtain meaningful data 300 animals are being studied and a minimum of 15,000 cells are being counted per tissue. Preliminary findings indicate that both the colchicine and tritiated thymidine methods yield similar data. Each show that the turnover rate of bronchial epithelial cells in mice is surprisingly low, ranging from 222 to 690 days. The turnover rates for mice with a high incidence of spontaneous lung tumors is, however, lower than that for mice exhibiting a low incidence of spontaneous lung tumors. There appears to be no difference in the turnover rates on the basis of the sex of the animals.

Tumor development in the cheek pouch of hamsters was achieved with low doses of 9,10-dimethylbenzanthracene which had been protected against UV radiation.

Cell-free homogenates of 3-methylcholanthrene-induced sarcomas enhanced tumor formation when applied with a chemical carcinogen. These results together with previous findings lends support to the theory that some host factor, perhaps a virus, is involved.

Ingestion of phenanthrene prior to feeding labeled benzo(a)pyrene to rats resulted in lower levels of radioactivity in the liver than in animals receiving no phenanthrene, indicating competition of these compounds in certain metabolic pathways. No change was found when phenol was administered one hour prior to the labeled benzo(a)pyrene.

Absorption and rate of transport in rat intestine of three strong carcinogens (benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenzanthracene) was greater than that for noncarcinogenic or weakly carcinogenic compounds. For benzo(a)pyrene the rate of transport into and across the intestinal mucosa increased exponentially with increase in concentration of the hydrocarbon in the medium. As the transport of benzo(a)pyrene was found to take place under anaerobic as well as aerobic conditions, the transport mechanism does not require energy in the same way as the intestinal transport of polar molecules (e.g. amino acids and sugars).

Components of an electron transport system in Bacteriodes melaninogenicus are flavoproteins, cytochrome C, a carbon monoxide binding pigment, and possibly cytochrome B. Exposure of the system to the carcinogen 2-n-nonyl-4-hydroxy-quinoline-N-oxide inhibited electron transport between the flavoprotein and cytochrome C and inhibited growth. Exposure to carbon monoxide, a component of tobacco smoke, also inhibited electron transport and growth.

Mouse strains prone to respiratory infections have a higher incidence of accelerated spontaneous tumors than infection-resistant strains. It therefore is important that the animal species used for assessing carcinogenicity via intranasal exposure be as free as possible from respiratory infection. The use of hamsters is being investigated since these animals are naturally resistant to infection and spontaneous lung tumors in these animals are extremely rare. Experiments are under way to establish techniques for intranasal inoculation which will assure particulate distribution to the terminal air passages.

Experiments to determine the optimum dose of an aqueous extract of cigarette smoke condensate (AECSC) which would be tolerated by rats and compatible with near optimum weight gains have been accomplished. The data obtained clearly show that for all groups treated with doses of AECSC higher than 0.5 mg/ml, dehydration and loss of body weight occurs. In one group, given 5 mg/ml smoke condensate, a significant degree of leucopenia occurred. Whether this was a consequence of decreased protein intake or a direct effect of the smoke extract could not be determined from the data. In this group all tissues of the reticuloendothelial system (liver, spleen, and thymus) were lighter than the controls on the basis of equal body weights. However, the kidneys, heart, lungs adrenals, and brain were heavier. Of the several concentrations of AECSC investigated, near optimal weight gains were achieved only at the 0.25 mg/ml dose level. This dose or one slightly lower will therefore be used in all future experiments.

The first phase of the contract bioassay of smoke fractions by Health Research Inc., Buffalo, New York, has been completed except for microscopic examination of tissues of the animals, still under way. The major carcinogenic substances of smoke condensate were found to occur in three of the 12 fractions tested: the weakly acidic (phenolic) fraction and two neutral sub-fractions (cyclohexane-soluble and nitromethane-soluble).

C. Microbiology and Fermentation

Phosphate esters of carbohydrates and nucleotides have shown promise as indicators of metabolic activity differences between fermented and cured tobaccos. A column chromatographic procedure was used in resolving various nucleoside mono-, di-, and tri-phosphates. Further separation and purification was achieved by a linear gradient procedure. This methodology has been applied in preliminary experiments to the analysis of tobacco extracts. Peroxidase activity in a 1967 Pennsylvania filler cigar tobacco sample decreased 40-60% during 14 weeks of curing. Cured filler tobacco samples dating back to the 1962 crop still had measurable, but slight, peroxidase activity. Techniques have been developed for the growth of Aspergillus niger on cigarette tobaccos. Under laboratory conditions tobacco infected with A. niger did not produce aflatoxin. Previously reported metabolic products in mold-infected tobacco were identical with nontoxic metabolites found earlier by other investigators.

D. Technology - Process and Product Development

1. Modification of Cigarette Burn Temperature. About 90 different additives were evaluated as burn temperature modifiers in contract work at the Houdry Laboratories, Air Products and Chemicals, Inc. Basic magnesium carbonate, nickel oxalate, aluminum sulfate octadecahydrate, and halides or carbonates of sodium, potassium, and silver depressed coal temperatures. Free radical initiators, such as benzothiazyl disulfide and benzyl disulfide, increased coal temperatures. In general, relatively large amounts of derivatives (20-50%) were required to alter burn temperatures markedly. It appears that additives effect changes in tobacco combustion products without significant modification of the coal temperature.

2. Production of Smoke Condensates and Experimental Cigarettes. Smoke condensate is prepared and fractionated at EU in kilogram quantities for bioassay research under contract with Health Research, Inc. Currently the condensate is separated into twelve fractions for use in animal tests.

At Lexington, Kentucky, production of smoke concentrate from machine-smoked cigarettes is now sufficient to meet the program requirements for whole tar and fractions thereof at that location. The condensates may be modified, as for example by the removal of nicotine, to meet the requirements of a particular end use.

In the bioassay service for the investigations at the University of Kentucky, experiments to assess the biological activity of cigarette smoke condensate from high- and low-nitrate burley tobaccos are in progress. To date, no tumors have appeared on the backs of mice painted with either non-denicotinized or denicotinized condensate. This includes 11 mice which have been painted with non-denicotinized, high-nitrogen burley condensate for 13 months. Of 100 mice (controls) painted with 0.005% benzo(a)pyrene for 13 months, 12 mice out of 37 survivors have developed tumors.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

A. Chemical Composition, Physical Properties and Structure

1. Cigar Smoke. The mainstream smoke condensate from one thousand cigars has been fractionated under mild conditions. Indications of dienes, trienes, and possibly tetraenes in a hydrocarbon fraction have been obtained. Although many of these compounds are present in small quantities they may have significant organoleptic properties.

2. Cigar Residual Aroma. Major volatile chemical constituents of cigar butts were identified as acetonitrile, propionitrile, acetone, butanone, dipentene, 3-acetylpyridine, pyrrole, nicotine, and water. Twelve other organic compounds, which were present in lower concentrations, could also contribute to the residual aroma of the four cigar filler types under investigation. Colombian tobacco had the highest concentration of isoprene in the mainstream

smoke. Puerto Rican was highest in acetaldehyde.

- B. Chemical and Physical Investigations to Improve Products (no current research)
- C. Microbiology and Fermentation (no current research)
- D. Technology - Process and Product Development (no current research)

Publications and Patents - USDA and Cooperative Programs

RPA 709 - REDUCTION IN HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

Chemical Composition, Physical Properties and Structure

- Cook, C. E., Twine, Margaret E. and Wall, M. E. 1967. Cholesteryl esters in flue-cured tobacco. *Experientia* 23, 987-989.
- Chortyk, O. T. 1967. Comparative studies on the brown pigments of tobacco. *Tob. Sci.*, 11, 137-139.
- Dymicky, M. and Stedman, R. L. 1967. Composition studies on tobacco. XXV. Moieties in a high molecular weight smoke pigment: alkaloids and a silicone. *Phytochem.*, 6, 1025-1031.
- Dymicky, M. and Stedman, R. L. 1968. Composition studies on tobacco. XXIX. Strongly acidic subfraction of a high molecular weight smoke pigment. *Tob. Sci.*, 12, 7-9.
- Krewson, C. F. and Chortyk, O. T. 1968. Comparative studies on tobacco leaf - use of a rapid extraction technique. *Am. J. Pharm.*, 140, 44-51.
- Miller, R. L. and Stedman, R. L. 1967. Essential absence of β -naphthylamine in cigarette smoke condensate. *Tob. Sci.*, 11, 111.
- Stedman, R. L., Miller, R. L., Lakritz, L. and Chamberlain, W. J. 1968. Method for concentrating polynuclear aromatic hydrocarbons in cigarette smoke condensate. *Chem. & Ind.* 394-395.
- Tucker, C. L. and Ogg, C. L. 1967. Determination of menthol in cigarette tobacco filler. *J. Assoc. Offic. Anal. Chemists*, 50, 770-773.
- Zane, A. 1968. Determination of anthracene and pyrene in cigarette smoke by gas chromatography. *Tob. Sci.* 12, 54-57.

Chemical and Physical Investigations to Improve Products

- Benner, J. F., Burton, H. R., and Burdick, D. 1968. Composition of cigarette smoke from high- and low-nitrate burley tobacco. *Tob. Sci.*, 12, 35-38.
- Burton, H. R., and Burdick, D. 1967. Thermal decomposition of tobacco. I. Thermogravimetric analysis. *Tob. Sci.* 11, 180-185.
- Humphrey, L. J. 1967. Studies on passage of hemadsorbing factor of murine tumors to normal cell cultures. *Cancer Res.*, 27, 2126-2128.
- Humphrey, L. J. 1967. Studies on the relationship of hemadsorption by normal murine spleen and murine tumors. *Cancer Res.*, 27, 2123-2125.

- Humphrey, L. J. 1967. Immunologic studies on the hemadsorbing factor of malignant tumors. Surgical Forum, 18, 94-96.
- Jones, T. C., and Schmeltz, I. 1968. Fingerprint gas chromatographic analysis of tobacco leaf acids. Tob. Sci., 12, 10-15.
- Schlotzhauer, W. S., and Schmeltz, I. 1967. Nonalkaloidal bases from pyrolysis of tobacco leaf pigment at the approximate burn temperature of a cigarette. Tob. Sci., 11, 89-90.
- Schmeltz, I., Hickey, L. C., and Schlotzhauer, W. S. 1967. Phenols from pyrolysis of tobacco leaf acids. Tob. Sci., 11, 52-53.

Microbiology and Fermentation

- Lerner, Joseph and Schepartz, Abner, I. 1968. Anion exchange chromatography of nucleotides. Fed. Proc., 27, No. 3, 3297 (Abstract).

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Chemical Composition, Physical Properties and Structure

- Osman, S. F., and Barson, J. L. 1967. Solvent fractionation of Girard T. derivatives of carbonyl compounds using dimethyl sulfoxide. Anal. Chem., 39, 530-531.
- Osman, S. and Barson, J. 1968. The chemical comparison of four cigar filler tobaccos. I. Preliminary investigation of mainstream and sidestream smoke. Tob. Sci., 12, 25-27.

MAPLE SAP AND SIRUP UTILIZATION

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968	
	: Research Problem Area	: Total
	: 401	:
Pennsylvania (Wyndmoor)	:	:
A. Chemical Composition and	:	:
Physical Properties	: 0.4	: 0.4
B. Microbiology and Toxicology	: 0.5	: 0.5
C. Technology - Process and	:	:
Product Development	: 2.4	: 2.4
	:	:
Total	: 3.3	: 3.3

Intramural program is supplemented by extramural support representing (a) 0 SMY's at universities and at state agricultural experiment stations, and (b) 0.1 SMY's at other U.S. institutions^{1/}.

^{1/} RPA 401 - Technology - Process and Product Development, 0.1.

Problems and Objectives

In the maple area, which includes 14 states from Maine to Minnesota and south to Virginia, only 3% of the available trees are tapped for sap production. Extensive unused stands of sugar maple trees are located in agriculturally depressed areas. Under proper conditions, maple sirup can be a six-weeks seasonal crop not in competition with other farm activities and with a per-acre value equal to or exceeding that of other farm products.

Emphasis is on obtaining the information needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner.

Major objectives of the research are:

1. Developing new technology to increase production.
2. Improving technology of sap collection and processing.
3. Improving quality of maple sirup and other maple products.
4. Developing new maple products.

Progress - USDA and Cooperative Programs

RPA 401 - NEW AND IMPROVED FOREST PRODUCTS

A. Chemical Composition and Physical Properties

When the flavor-containing chloroform extract of maple sirup was subjected to a gas liquid chromatograph (GLC)-mass spectrograph tandem procedure, 35 compounds were identified. Most of the compounds are present in very low concentrations. The maple flavor constituents or their derivatives are considered to derive from two sources; lignin or lignin-like materials, and sugars and their degradation products. Other compounds have apparently been introduced into the extract as impurities from the different solvents or as contaminants from the anti-foaming agents added in processing sap to sirup.

A GLC procedure has been developed to obtain from the flavor-containing extract of maple sirup a single "profile" type chromatogram. The "maple flavor profiles" can be compared to show effects of sap source and processing and storage conditions on maple flavor.

A stock of freeze-dried sap solids is being accumulated for use in the flavor precursor isolation and identification studies. Reverse osmosis provides an excellent means for the partial concentration of sap prior to freeze-drying. Concentrating the sap at or below room temperature prevents damage to heat labile substances and minimizes loss of volatile components in the sap. Initial work on the identity of the flavor precursors, however, indicates their nonvolatile nature.

This fundamental research on flavor constituents and precursors is needed to evaluate the effect of technological changes on quality of maple products.

B. Microbiology and Toxicology

Time-temperature studies of effectiveness of ultraviolet irradiation on control of microbial growth in sap showed that a longer irradiation time is necessary as temperature is lowered from 60° to 35°F. Exposure of recirculated sap to the irradiation reduced viable cell counts in all samples: 35°F., 55% reduction in 6 hours; 45°F., 95% in 4 hours and at 65°F., 95% in 1-1/2 hours.

Acidified hypochlorite followed by rinsing with ultraviolet-radiated water was found effective in sanitizing reverse osmosis equipment. The importance of sanitizing sap collecting equipment and hauling tanks was confirmed.

A fermentation process for eliminating "buddy" flavor from sap and from finished sirup was successfully field-tested on 2500 gallons of sap.

C. Technology - Process and Product Development

1. Maple Sap Storage. Field studies showed that maple sap, irradiated with ultraviolet light before storage and held under continuous irradiation, made as good sirup after 6 days as originally; a control yielded sirup 2 grades darker.

Continuous irradiation of recirculated sap during storage controlled the microbial count almost as well as the preceding treatment. Sap treated only by ultraviolet irradiation while flowing into the storage tank with no subsequent treatment during storage had higher yeast and mold counts and the sirup made at the end of the storage period was a full grade darker than that made from the original sap.

These results confirm those obtained in previous years under the contract, now completed, with J. L. Sipple and Son, Bainbridge, New York. Ultraviolet irradiation of stored sap will be recommended for use in central evaporator plant operations.

2. Sap Concentration by Reverse Osmosis. Reverse osmosis concentrating equipment was assembled and tested in preparation for field use during 1968 maple sap season. Test data indicate that the equipment will remove 60% of the water that must be removed in concentrating maple sap to sirup.

Publications and Patents - USDA and Cooperative Programs

RPA 401 - NEW AND IMPROVED FOREST PRODUCTS

Chemical Composition and Physical Properties

Gallander, J. F., Hacskeylo, J., Gould, W. A., and Willits, C. O. 1967. Environmental and chemical factors associated with maple sugar sand formation. Ohio Agri. Res. & Dev. Center, Res. Bull. 999.

Microbiology and Toxicology

Kissinger, J. C. 1967. Determining the yeast count in maple sirup and a collaborative study of the methods and sampling techniques. J. Assoc. Offic. Anal. Chemists, 50, 747-751.

Technology - Process and Product Development

Willits, C. O. 1968. A completely automatic sirup draw-off. Nat. Maple Syrup Dig., 7, no. 1, 12-13.

Willits, C. O. 1968. Soft sugar cakes. Nat. Maple Syrup Dig., 7, no. 1, 8-9.

Willits, C. O. and Underwood, J. S. December 5, 1967. Continuous process for high-flavoring maple sirup. U. S. Patent 3,356,508.

PUBLICATIONS - DISCONTINUED REPORTING AREA

White, J. W., Jr. 1967. Measuring honey quality - a rational approach. Am. Bee J., 107, 374-375.

White, J. W., Jr. 1967. Moisture determination in honey with an Eichhorn-type hydrometer. J. Apicult. Res., 6, 11-16.

White, J. W., Jr. 1967. Honey, its composition and properties. U. S. Dept. Agr., Agricultural Handbook No. 335, 56-64.

White, J. W., Jr. and Kushnir, Irene. 1967. The enzymes of honey: examination by ion exchange chromatography, gel filtration, and starch-gel electrophoresis. J. Apicult. Res., 6, 69-89.

White, J. W., Jr. and Kushnir, Irene. 1967. Composition of honey. VII. Proteins. J. Apicult. Res., 6, 163-178.

